WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(54) Title: NUCLEIC ACID SEQUENCES ENCODING A HUMAN DOPAMINE D_1 RECEPTOR

(57) Abstract

This invention provides isolated nucleic acid molecules encoding a human dopamine D_1 receptor, isolated proteins which are human dopamine D_1 receptor, vectors comprising isolated nucleic acid molecules encoding a human dopamine D_1 receptor, mammalian cells comprising such vectors, antibodies directed to a human dopamine D_1 receptor, nucleic acid probes useful for detecting nucleic acid encoding human dopamine D_1 receptor, antisense oligonucleotides complementary to any sequences of a nucleic acid molecule which encodes a human dopamine D_1 receptor, pharmaceutical compounds related to human dopamine D_1 receptor, and nonhuman transgenic animals which express DNA a normal or a mutant human dopamine D_1 receptor. This invention further provides methods for determining ligand binding, detecting expression, drug screening, and treatment involving a human dopamine D_1 receptor.

d)

"Nucleic Acid Sequences Encoding a Human Dopamine D_1 Receptor".

5

This application is a continuation-in-part of U.S. Serial No. 551,448, filed July 10, 1990, the contents of which are hereby incorporated by reference into the present disclosure.

10

Throughout this application various publications are referenced by full citations within parentheses. The disclosures of these publications in their entireties are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains.

15

Pharmacological studies, and more recently gene cloning, have established that multiple receptor subtypes exist for most, if not all, neurotransmitters. The existence of multiple receptor subtypes provides one mechanism by which a single neurotransmitter can elicit distinct cellular responses. The variation in cellular response can be achieved by the association of individual receptor subtypes with different G proteins and different signalling systems. Further flexibility is provided by the ability of distinct receptors for the same ligand to activate or inhibit the same second messenger system.

20

25

30

Individual receptor subtypes reveal characteristic differences in their abilities to bind a number of ligands, but the structural basis for the distinct ligand-binding properties is not known. Physiologists and pharmacologists

35

have attempted to specify particular biological functions or anatomical locations for some receptor subtypes, but this has met with limited success. Similarly, the biochemical mechanisms by which these receptors transduce signals across the cell surface have been difficult to ascertain without having well-defined cell populations which express exclusively one receptor subtype.

Dopamine receptors have been classified into two subtypes, D_1 and D_2, based on their differential affinities for dopamine agonists and antagonists, and their stimulation or inhibition of adenylate cyclase (for reviews, see Kebabian, J.W. and Calne, D.B. (1979), Nature 277, 93-96; Creese, I., Sibley, D.R., Hamblin, M.W., Leff, S.E. (1983), Ann. Rev. Neurosci. 6, 43-71; Niznik, H.B. and Jarvie, K.R. (1989), Dopamine receptors. in "Receptor Pharmacology and Function", eds. Williams, M., Glennon, R., and Timmermans, P., Marcel Dekker Inc., New York, pp. 717-768). The D_1 receptor of the central nervous system is defined as an adenylate cyclase stimulatory receptor. The location of the prototypic D_1 receptor is the bovine parathyroid gland, where dopamine agonists stimulate cAMP synthesis via adenylate cyclase, accompanied by parathyroid hormone release. Dopamine-stimulated adenylate cyclase activity and parathyroid hormone release are sensitive to both GTP and cholera toxin. This suggests that the D_1 receptor is associated with a G_s guanine nucleotide binding protein. The D_2 receptor, in contrast, inhibits adenylate cyclase activity, and appears to be the primary target of most neuroleptic drugs (Niznik, H.B. and Jarvie, K.R. (1989). Dopamine receptors, in "Receptor Pharmacology and Function", eds. Williams, M., Glennon, R., and Timmermans, P., Marcel Dekker Inc., New

York, pp. 717-768). The prototypic D_2 receptor has been characterized in the anterior pituitary where it is associated with the inhibition of release of prolactin and alpha-melanocyte stimulating hormones. Recent wo: has shown that several different D_1 and D_2 receptor subtypes may be present in the mammalian nervous system (Andersen, P.H., Gingrich, J.A., Bates, M.D., Dearry, A., Falardeau, P., Senogles, S.E., and Caron, M.G. Trends in Pharmacolog. Sci. 11: 231 (1990)), which would suggest that a family of different proteins with pharmacological properties similar to the classically defined D_1 and D_2 receptors may exist.

Neuroleptics, in addition to their use as drugs to treat severe psychiatric illnesses, are high affinity ligands for dopamine receptors. Butyrophenones such as haloperidol and spiperone are antagonists specific for the D_2 receptor, while the recently developed benzazepines such as SCH-23390 and SKF-38393 are selective for the D_1 receptor (Niznik, H.B. and Jarvie, K.R. (1989), Dopamine receptors, in "Receptor Pharmacology and Function", eds. Williams, M., Glennon, R., and Timmermans, P., Marcel Dekker Inc., New York, pp. 717-768). High affinity D_1 and D_2 selective ligands have conclusively distinguished these receptors and made feasible characterization of the receptors in the central nervous system and peripheral tissues with radioligand binding techniques. Two types of dopamine receptors, designated D_{A1} and D_{A2}, have been identified in the cardiovascular system and are similar in their pharmacological characteristics to the brain D_1 and D_2 receptors (Niznik, H.B. and Jarvie, K.R. (1989), Dopamine receptors, in "Receptor Pharmacology and Function", eds. Williams, M., Glennon, R., and Timmermans, P., Marcel Dekker Inc., New York, pp. 717-768). D_{A1} receptors have been

described in renal, mesenteric, splenic, coronary, cerebral, and pulmonary arteries and vascular beds, where dopamine elicits relaxation of vascular smooth muscle. Activation of cardiovascular D_{A1} receptors appears to stimulate adenylate cyclase activity. D_{A2} receptors appear to be localized on preganglionic sympathetic nerve terminals that mediate inhibition of norepinephrine release. The molecular relationships among dopamine D_1, D_{A1}, D_2, and D_{A2} receptors are unknown.

The need for improved selectivity in the leading D_1 drug class, the benzazepines (e.g. SKF-38393, SCH-23390 and SCH-23982) recently became apparent when the strong cross-reactivity of these drugs with the serotonin $5-HT_2$ receptor family was uncovered. The $5-HT_2$ and $5-HT_{1c}$ receptors display affinities ranging from 0.2 to 24 nM for SCH-23390 and SCH-23982 (Nicklaus, K.J., McGonigle, P., and Molinoff, P.B. (1988), J. Pharmacol. Exp. Ther. 247, 343-348; Hoyer, D. and Karpf, A. (1988), Eur. J. Pharmacol. 150, 181-184)), raising the possibility that behavioral and pharmacological effects ascribed to these drugs may, in fact, arise from serotonergic receptor interactions.

The dopamine D_1 receptors belong to a family of receptors which are distinguished by their seven-transmembrane configuration and their functional linkage to G-proteins. This family includes rhodopsin and related opsins (Nathans, J. and Hogness, D.S., Cell 34:807 (1983)), the α and β adrenergic receptors (Dohlman, H.G., et al., Biochemistry 26:2657 (1987)), the muscarinic cholinergic receptors (Bonner, T.I., et al., Science 237:527 (1987)), the substance K neuropeptide receptor, (Masu, Y., et al., Nature 329:836 (1987)), the yeast mating factor receptors,

(Burkholder, A.C. and Hartwell, L.H., Nucl. Acids Res. 13:8463(1985); Hagan, D.C., et al., Proc. Natl. Acad. Sci. USA 83:1418 (1986)); Nakayama, N. et al., EMBO J. 4:2643 (1985)), and the oncogene c-mas, (Young, et al., Cell 45:711 (1986)). Each of these receptors is thought to transduce extracellular signals by interaction with guanine nucleotide-binding (G) proteins (Dohlman, H.G., et al., Biochemistry 26:2657 (1987); Dohlman, H.G., et al., Biochemistry 27:1813 (1988); O'Dowd, B.F., et al., Ann.Rev. Neurosci., in press).

The D_2 receptor was recently cloned by Civelli and colleagues (Bunzow, J.R., Van Tol, H.H.M., Grandy, D.K., Albert, P., Salon, J., Christie, M., Machida, C.A., Neve, K.A., and Civelli, O. (1989), Nature 336: 783-87). This event was soon followed by the discovery of an alternatively spliced form (termed D_{2A}, D_{2long}, $D-2_{in}$, or $D_{2(444)}$) that contains an additional 29 amino acids in the third extracellular loop of this receptor (Eidne, K.A. et al. (1989), Nature 342: 865; Giros, B. et al. (1989), Nature 342: 923-26; Grandy, D.K. et al. (1989), Proc. Natl. Acad. Sci. USA 86: 9762-66; Monsma, F.J. et al. (1989), Nature 342: 926-29; Chio, C.L. et al. (1990), Nature 343: 266-69; Stormann, T.M. et al. (1990), Mol. Pharmacol. 37: 1-6). A second dopamine receptor has been cloned which exhibits significant homology to the D_2 receptor, both in amino acid sequence (75% transmembrane region identity) and in pharmacological properties (Sokoloff, P. et al. (1990), Nature 347: 146-51). This new receptor, termed D_3, is encoded by an intron-containing gene. Unlike the D_2 receptor, however, alternatively spliced isoforms of this receptor have yet to be observed. The D_3 receptor has been shown to serve both as an autoreceptor and as a postsynaptic

receptor, and has been localized to limbic areas of the brain (Sokoloff, P. et al. (1990), Nature 347: 146-51). Finally, an intronless gene, quite different in sequence and gene structure from the other two dopamine receptor genes, has been isolated and identified as a D_1 dopamine receptor subtype (Sunahara, R.K. et al. (1990), Nature 347: 80-83; Zhou, Q.-Y. et al. (1990), Nature 347: 76-80; Dearry, A. et al. (1990), Nature 347: 72-76; Monsma, F.J. et al. (1990), Proc. Natl. Acad. Sci. USA 87: 6723-27). This D_1 receptor is predominantly expressed in the rat striatum and olfactory tubercles, and has been shown to couple to stimulation of adenylate cyclase activity (Dearry et al. (1990) supra; Monsma et al. (1990) supra; Sunahara et al. (1990) supra; Zhou et al. (1990) supra. Available data on the G protein-coupled receptor superfamily suggests that the D_1 receptor does not exhibit strong sequence homologies to the D_2 receptor or the D_3 receptor. In general, G protein-coupled receptors of the same neurotransmitter family exhibit closest structural homology to other family members that use the same second messenger pathway. For example, examination of the physiological second messenger pathways of the serotonergic, muscarinic and adrenergic receptors has led several researchers to the conclusion that these receptors can be classified into structurally homologous subtypes that parallel their second messenger pathways (Bylund, D.B. (1988), Trends Pharmacol. Sci. 9, 356-361; Peralta, E.G., Ashkenazi, A., Winslow, J.W., Ramachandran, J., and Capon, D.J. (1988), Nature 334, 434-437; Liao, C.-F., Themmen, A.P.N., Joho, R., Barberis, C., Birnbaumer, M., and Birnbaumer, L. (1989), J. Biol. Chem. 264, 7328-7337; Hartig, P.R. (1989), Trends Pharmacol. Sci. 10, 64-69)). Interestingly, those receptors that couple to activation of adenylate cyclase appear quite distinct in structure from those that inhibit this enzyme activity.

pharmacological and physiological data have emerged indicating the presence of further diversity within this receptor family. A D_1 receptor that stimulates phosphoinositide (PI) hydrolysis in rat striatum has been described (Undie, A.S., and Friedman, E. (1990), J. Pharmacol. Exp. Ther. 253: 987-92) as well as an RNA fraction from the same tissue that causes dopamine-stimulated PI hydrolysis and intracellular calcium release when injected into Xenopus oocytes (Mahan, L.C. et al. (1990), Proc. Natl. Acad. Sci. USA 87: 2196-2200). In addition, two populations of peripheral D_1 receptor have been described based on differential sensitivity to sulpiride and several other compounds (Andersen, P.H. et al. (1990), Eur. J. Pharmacol. 137: 291-93). Finally, pharmacological differences exist within different D_1 receptor tissues that couple to adenylate cyclase-coupled D_1 receptors. Biochemical and pharmacological data suggest further diversity in both the D_1 and D_2 receptor populations and indicate that additional dopamine receptor clones remain to be discovered (Andersen et al. (1990) _supra_).

Summary of the Invention

This invention provides an isolated nucleic acid molecule encoding a human dopamine D_1 receptor.

This invention also provides an isolated protein which is a human dopamine D_1 receptor, an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1.

This invention provides a vector comprising an isolated nucleic acid molecule encoding a human dopamine D_1 receptor.

This invention provides a mammalian cell comprising a DNA molecule encoding a human dopamine D_1 receptor.

This invention provides a method for determining whether a ligand not known to be capable of binding to a human dopamine D_1 receptor can bind to a human dopamine D_1 receptor which comprises contacting a mammalian cell comprising a DNA molecule encoding a human dopamine D_1 receptor with the ligand under conditions permitting binding of ligands known to bind to the dopamine D_1 receptor, detecting the presence of any of the ligand bound to the dopamine D_1 receptor, and thereby determining whether the ligand binds to the dopamine D_1 receptor.

This invention also provides a method of screening drugs to identify drugs which specifically interact with, and bind to, the human dopamine D_1 receptor on the surface of a cell which comprises contacting a mammalian cell comprising a DNA molecule encoding a human dopamine D_1 receptor on the surface of a cell with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby

identifying drugs which specifically interact with, and bind to, the human dopamine D_1 receptor.

5   This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human dopamine D_1 receptor.

10   This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a human dopamine D_1 receptor so as to prevent translation of the mRNA molecule.

15
This invention provides an antibody directed to the human dopamine D_1 receptor.

This invention provides a transgenic nonhuman mammal
20   expressing DNA encoding a human dopamine D_1 receptor. This invention also provides a transgenic nonhuman mammal expressing DNA encoding a human dopamine D_1 receptor so mutated as to be incapable of normal receptor activity, and not expressing native dopamine D_1 receptor. This invention
25   further provides a transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a human dopamine D_1 receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a dopamine D_1 receptor and which hybridizes to mRNA encoding
30   a dopamine D_1 receptor thereby reducing its translation.

This invention provides a method of determining the physiological effects of expressing varying levels of human

dopamine D_1 receptors which comprises producing a transgenic nonhuman animal whose levels of human dopamine D_1 receptor expression are varied by use of an inducible promoter which regulates human dopamine D_1 receptor expression.

This invention also provides a method of determining the physiological effects of expressing varying levels of human dopamine D_1 receptors which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human dopamine D_1 receptor.

This invention provides a method for diagnosing in a subject a predisposition to a disorder associated with the expression of a specific human dopamine D_1 receptor allele which comprises a. isolating DNA from victims of the disorder, b. digesting the isolated DNA of step a with at least one restriction enzyme, c. electrophoretically separating the resulting DNA fragments on a sizing gel, d. contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human dopamine D_1 receptor and labelled with a detectable marker, e. detecting labelled bands which have hybridized to the DNA encoding a human dopamine D_1 receptor labelled with a detectable marker to create a band pattern specific to the DNA of victims of the disorder, f. preparing the subject's DNA by steps a-e to produce detectable labeled bands on a gel, and g. comparing the band pattern specific to the DNA of victims of the disorder of step e and the subject's DNA of step f to determine whether the patterns are the same or different and thereby to diagnose predisposition to the disorder if the patterns are the same. This method may also be used to diagnose a disorder associated with the expression of a specific human dopamine D_1 receptor allele.

-11-

This invention provides a method of preparing the isolated dopamine D_1 receptor which comprises inducing cells to express dopamine D_1 receptor, recovering the receptor from the resulting cells, and purifying the receptor so recovered.

This invention provides a method of preparing the isolated dopamine D_1 receptor which comprises inserting nucleic acid encoding dopamine D_1 receptor in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the receptor produced by the resulting cell, and purifying the receptor so recovered.

Brief Description of the Figures

Figure 1. Nucleotide and deduced amino acid sequence of the gene GL-30. (Also Seq. ID No. 1).

5

Numbers above the nucleotide sequence indicate nucleotide position.   DNA sequence was determined by the chain termination method of Sanger, et al., on denatured doubled-stranded plasmid templates using the enzyme Sequenase. Deduced amino acid sequence (single letter code) of a long

10

open reading frame is shown.

Figure 2. Comparison of the Dopamine D_1 (GL-30) receptor primary structure with other G-protein-coupled receptors. Amino acid sequences (single letter code) are aligned to

15

optimize homology. GL-30 is the human dopamine receptor of this invention; GL-39 is the human dopamine pseudogene; and D_1 is the human dopamine D_1 receptor. (Also Seq. ID Nos. 2 to 4, respectively).   It should be noted that a clone

20

designated D_5 is the same sequence as that listed as GL-30. (Sunahara, et al. (April 1991) Nature, 350:614-619).

-13-

Detailed Description of the Invention

As used herein, the dopamine receptor family is defined as
the group of mammalian proteins that function as receptors
for dopamine. A dopamine receptor subfamily is defined as
a subset of proteins belonging to the dopamine receptor
family which are encoded by genes which exhibit homology of
65% or higher with each other in their deduced amino acid
sequences within presumed transmembrane regions (linearly
contiguous stretches of hydrophobic amino acids, bordered by
charged or polar amino acids, that are long enough to form
secondary protein structures that span a lipid bilayer).
Three   human   dopamine   receptor   subfamilies   can   be
distinguished based on the information presently available.
The dopamine D_2 receptor subfamily contains the dopamine D_2
receptor. There are currently two forms of this receptor
which are generated by alternative splicing mechanisms
(Toso, R.D., Sommer,B, Ewert, M, et al. (1989) EMBO 8:4025-
4034; Chio, C.L. et al. (1990) Nature 343:266-269; Monsma,
F.J. (1990) Nature 342:926-929). The dopamine D_3 receptor
which exhibits significant homology to the D_2 receptor both
in amino acid sequence and pharmacological properties
(Sokoloff, P. et al. (1990) supra). The human dopamine D_1
receptor subfamily contains the human dopamine D_1 receptor
gene GL-30 which is described herein, and the human dopamine
D_1 receptor, not yet cloned or isolated, which represents
the human counterpart of the rat D_1 clone (Sunahara R.K.
(1990) Nature 347:80-83).   Therefore, the term "human
dopamine D_1 receptor" as used herein is defined as meaning
a member of the dopamine D_1 receptor subfamily described
above.   Although this definition differs from the
pharmacological definition used earlier, there is
significant overlap between the present definition and the

pharmacological definition. Members of the human dopamine D_1 receptor subfamily so described include the dopamine D_1 receptor clone known as GL-30 (which is also known as dopamine $D_{1\beta}$ receptor subtype) and any other receptors which have a 65% or greater transmembrane homology to the DNA and amino acid sequence shown in Figure 1 according to the definition of "subfamily". This invention relates to the discovery of the first member of the human dopamine D_1 receptor subfamily.

This invention provides an isolated nucleic acid molecule such as a DNA molecule encoding a human dopamine D_1 receptor. Such a receptor is by definition a member of the dopamine D_1 receptor subfamily. Therefore, any receptor which meets the defining criteria given above is a human dopamine D_1 receptor. One means of isolating a human dopamine D_1 receptor is to probe a human genomic library with a natural or artificially designed DNA probe, using methods well known in the art. DNA probes derived from the human genes encoding dopamine D_1 receptor, for example clone GL-30 is a particularly useful probe for this purpose. DNA and cDNA molecules which encode human dopamine D_1 receptors may be used to obtain complementary genomic DNA, cDNA or RNA from human, mammalian or other animal sources, or to isolate related cDNA or genomic clones by the screening of cDNA or genomic libraries, by methods described in more detail below. Transcriptional regulatory elements from the 5' untranslated region of the isolated clones, and other stability, processing, transcription, translation, and tissue specificity-determining regions from the 3' and 5' untranslated regions of the isolated genes are thereby obtained. Examples of a nucleic acid molecule are an RNA, cDNA, or isolated genomic DNA molecule encoding a human

dopamine D_1 receptor. Such molecules may have coding sequences substantially the same as the coding sequence shown in Figure 1 or may have coding sequences that are 65% or more homologous to the coding sequence shown in Figure 1.

5   The DNA molecule of Figure 1 encodes a human dopamine D_1 receptor.

This invention further provides a cDNA molecule encoding a human dopamine D_1 receptor having a coding sequence

10   substantially the same as the coding sequence shown in Figure 1. This molecule is obtained by the means described above.

This invention also provides an isolated protein which is a

15   human dopamine D_1 receptor. Examples of such proteins are an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1, which is a human dopamine D_1 receptor. One means for obtaining isolated dopamine D_1 receptor is to express DNA encoding the

20   receptor in a suitable host, such as a bacterial, yeast, or mammalian cell, using methods well known in the art, and recovering the receptor protein after it has been expressed in such a host, again using methods well known in the art. The receptor may also be isolated from cells which express

25   it, in particular from cells which have been transfected with the expression vectors described below in more detail.

This invention provides a vector comprising an isolated nucleic acid molecule such as DNA, RNA, or cDNA encoding a

30   human dopamine D_1 receptor. Examples of vectors are viruses such as bacteriophages (such as phage lambda), cosmids, plasmids (such as pUC18, available from Pharmacia, Piscataway, NJ), and other recombination vectors. Nucleic

acid molecules are inserted into vector genomes by methods well known in the art. For example, insert and vector DNA can both be exposed to a restriction enzyme to create complementary ends on both molecules which base pair with
5   each other and are then ligated together with a ligase. Alternatively, linkers can be ligated to the insert DNA which correspond to a restriction site in the vector DNA, which is then digested with the restriction enzyme which cuts at that site. Other means are also available. An
10   example of a plasmid is a plasmid comprising DNA having a coding sequence substantially the same as the coding sequence shown in Figure 1 and designated clone pdopD1-GL-30, deposited with the American Type Culture Collection under ATCC Accession No. 40839.

15
This deposit was made pursuant to, and in satisfaction of, the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure with the American Type Culture Collection (ATCC),
20   12301 Parklawn Drive, Rockville, Maryland 20852.

This invention also provides vectors comprising a DNA molecule encoding a human dopamine D_1 receptor adapted for expression in a bacterial cell, a yeast cell, or a mammalian
25   cell which additionally comprise the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cells so located relative to the DNA encoding a human dopamine D_1 receptor as to permit expression thereof. DNA having coding sequences substantially the same
30   as the coding sequence shown in Figure 1 may usefully be inserted into the vectors to express human dopamine D_1 receptors. Regulatory elements required for expression include promoter sequences to bind RNA polymerase and

transcription initiation sequences for ribosome binding.
For example, a bacterial expression vector includes a
promoter such as the lac promoter, and for transcription
initiation, the Shine-Dalgarno sequence and the start codon
ATG (Maniatis, et al., Molecular Cloning, Cold Spring Harbor
Laboratory, 1982). Similarly, a eucaryotic expression
vector includes a heterologous or homologous promoter for
RNA polymerase II, a downstream polyadenylation signal, the
start codon ATG, and a termination codon for detachment of
the ribosome. Such vectors may be obtained commercially or
assembled from the sequences described by methods well known
in the art, for example the methods described above for
constructing vectors in general. Expression vectors are
useful to produce cells that express the receptor. Certain
uses for such cells are described in more detail below.

This invention further provides a plasmid adapted for
expression in a bacterial, yeast, or, in particular, a
mammalian cell which comprises a DNA molecule encoding a
human dopamine D_1 receptor and the regulatory elements
necessary for expression of the DNA in the bacterial, yeast,
or mammalian cell so located relative to the DNA encoding a
human dopamine D_1 receptor as to permit expression thereof.
Some plasmids adapted for expression in a mammalian cell are
pSVL (available from Pharmacia, Piscataway, NJ) and pcEXV-3
(Miller J. and Germain R.N., J. Exp. Med. 164:1478 (1986)).
Specific examples of such plasmids are a plasmid adapted for
expression in a mammalian cell comprising cDNA having coding
sequences substantially the same as the coding sequence
shown in Figure 1 and the regulatory elements necessary for
expression of the DNA in the mammalian cell. Those skilled
in the art will readily appreciate that numerous plasmids
adapted for expression in a mammalian cell which comprise

DNA encoding human dopamine D_1 receptors and the regulatory elements necessary to express such DNA in the mammalian cell may be constructed utilizing existing plasmids and adapted as appropriate to contain the regulatory elements necessary to express the DNA in the mammalian cell. The plasmids may be constructed by the methods described above for expression vectors and vectors in general, and by other methods well known in the art.

This invention provides a mammalian cell comprising a DNA molecule encoding a human dopamine D_1 receptor, such as a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, which comprises a DNA molecule encoding a human dopamine D_1 receptor and the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding a human dopamine D_1 receptor as to permit expression thereof. Numerous mammalian cells may be used as hosts, including the mouse fibroblast cell NIH3T3, CHO cells, HeLa cells, and Ltk-cells. Expression plasmids such as those described supra may be used to transfect mammalian cells by methods well known in the art such as calcium phosphate precipitation, or DNA encoding these dopamine D_1 receptors may be otherwise introduced into mammalian cells, e.g., by microinjection, to obtain mammalian cells which comprise DNA, e.g., cDNA or a plasmid, encoding a human dopamine D_1 receptor.

This invention provides a method for determining whether a ligand not known to be capable of binding to a human dopamine D_1 receptor can bind to a human dopamine D_1 receptor which comprises contacting a mammalian cell comprising a DNA molecule encoding a human dopamine D_1 receptor with the ligand under conditions permitting binding

-19-

of ligands known to bind to the dopamine D_1 receptor, detecting the presence of any of the ligand bound to the dopamine D_1 receptor, and thereby determining whether the ligand binds to the dopamine D_1 receptor. Methods for
5 performing this technique are well known in the art. The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figure 1. Preferably, the mammalian cell is nonneuronal in origin. An example of a nonneuronal mammalian cell is an Ltk- cell. (Stable cell
10 lines can be produced by cotransfection of an expression plasmid such as pSVL or pcEXV, into which the DNA of Figure 1 has been subcloned, with a plasmid containing the bacterial gene aminoglycoside phosphotransferase into Ltk-cells (American Type Culture Collection, Rockville, MD, Cell
15 Line CCL 1,3) using the calcium phosphate technique (protocol & kit obtained from Specialty Media, Inc. Lavallette, NJ). Clones expressing aminoglycoside transferase can then be selected by the addition of 1 mg/ml G418 (Gibco Laboratories, Grand Island, NY) to the culture
20 medium. The preferred method for determining whether a ligand is capable of nding to the human dopamine D_1 receptors comprises contacting a transfected nonneuronal mammalian cell (i.e. a cell that does not naturally express any type of dopamine or G-protein coupled receptor, thus
25 will only express such a receptor if it is transfected into the cell) expressing a dopamine D_1 receptor on its surface, or contacting a membrane preparation derived from such a transfected cell, with the ligand under conditions which are known to prevail, and thus to be associated with, in vivo
30 binding of the ligands to a dopamine D_1 receptor, detecting the presence of any of the ligand being tested bound to the dopamine D_1 receptor on the surface of the cell, and thereby determining whether the ligand binds to the dopamine D_1

receptor. (Methods for so doing are well known in the art, for example a tritiated ligand can be used as a radioligand to detect binding to membrane fractions isolated from either transiently or stably transfected cell lines which express human dopamine D_1 receptor. Tritiated SCH-23390 (71.3 Ci/mMol; Dupont-NEN), which is known in the art to bind with high affinity to the dopamine D_1 receptor, is used as a radioligand to detect the expression of the dopamine D_1 gene product in membrane fractions isolated from either transiently or stably transfected cell lines. The incubation buffer contains 50 mM Tris-HCl pH 7.4; 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$; 2mM $CaCl_2$; 0.1% ascorbic acid; and 1 μM pargyline, and incubation is initiated by adding cell membranes 10-50 μg/well to a 96 well microtiter plate containing tritiated SCH-23390 (final concentration 5 nM) in a final volume of 250 μl. After incubating 20 minutes at 37°C in the dark, incubation is terminated by rapid filtration with a Brandel Model 48R Cell Harvester (Brandel, Gaithersville, MD). The tritiated SCH-23390 that has bound to the dopamine receptors on the cell membrane is retained on the filters, which are placed in scintillation vials with a scintillation fluid (such as Ready Safe, Beckman Instruments, Fullerton, CA) and counted in a scintillation counter (such as a Beckman LS5000 TA). Specific binding of tritiated SCH-23390 is determined by defining nonspecific binding with 10^{-6} M cis(-) flupentixol. To determine whether a ligand binds to dopamine D_1 receptor, the ligand can be tritiated by methods well known in the art, and the technique described above for SCH-23390 binding performed. But a more efficient method is to perform competition studies. The method described above is performed, however in addition to tritiated SCH-23390, a different unlabeled ligand is added to each well of the incubation except

control wells.   Ligands are initially screened at a
concentration of 1-10 times their reported Ki values for
dopamine D_1 receptor binding by liquid scintillation
spectroscopy in a Beckman LS5000 TA scintillation counter
using Ready Safe liquid scintillation cocktail (Beckman
Instruments, Fullerton, CA) at an efficiency of 50-55%.
Whichever ligand reduces the counts of radioactivity over
the counts of tritiated SCH-23390 alone has competitively
reduced binding of the tritiated SCH-23390 by itself binding
to the dopamine receptor). This response system is obtained
by transfection of isolated DNA into a suitable host cell
containing the desired second messenger system such as
phosphoinositide hydrolysis, adenylate cyclase, guanylate
cyclase or ion channels. Such a host system is isolated
from pre-existing cell lines, or can be generated by
inserting appropriate components of second messenger systems
into existing cell lines.   Such a transfection system
provides a complete response system for investigation or
assay of the activity of human dopamine D_1 receptors with
ligands as described above.   Transfection systems are
useful as living cell cultures for competitive binding
assays between known or candidate drugs and ligands which
bind to the receptor and which are labeled by radioactive,
spectroscopic or other reagents.   Membrane preparations
containing the receptor isolated from transfected cells are
also useful for these competitive binding assays.
Functional assays of second messenger systems or their
sequelae in transfection systems act as assays for binding
affinity and efficacy in the activation of receptor
function.   A transfection system constitutes a "drug
discovery system" useful for the identification of natural
or synthetic compounds with potential for drug development
that can be further modified or used directly as therapeutic

compounds to activate or inhibit the natural functions of
the human dopamine D_1 receptor. The transfection system is
also useful for determining the affinity and efficacy of
known drugs at the human dopamine D_1 receptor sites.

This invention also provides a ligand detected by the method
described supra.

This invention also provides a method of screening drugs to
identify drugs which specifically interact with, and bind
to, the human dopamine D_1 receptor on the surface of a cell
which comprises contacting a mammalian cell comprising a DNA
molecule encoding a human dopamine D_1 receptor on the
surface of a cell with a plurality of drugs, determining
those drugs which bind to the mammalian cell, and thereby
identifying drugs which specifically interact with, and bind
to, the human dopamine D_1 receptor. The DNA in the cell may
have a coding sequence substantially the same as the coding
sequence shown in Figure 1. Preferably, the mammalian cell
is nonneuronal in origin, such as an Ltk- cell. Drug
candidates are identified by choosing chemical compounds
which bind with high affinity to the expressed dopamine D_1
receptor protein in transfected cells, using radioligand
binding methods well known in the art and described supra.
Drug candidates are also screened for selectivity by
identifying compounds which bind with high affinity to one
particular dopamine receptor but do not bind with high
affinity to any other dopamine receptor subtype or to any
other known receptor site. Because selective, high affinity
compounds interact primarily with the target dopamine D_1
receptor site after administration to the patient, the
chances of producing a drug with unwanted side effects are
minimized by this approach.   This invention provides a

pharmaceutical composition comprising a drug identified by the method described above and a pharmaceutically acceptable carrier. Once the candidate drug has been shown to be adequately bio-available following a particular route of administration, for example orally or by injection (adequate therapeutic concentrations must be maintained at the site of action for an adequate period to gain the desired therapeutic benefit), and has been shown to be non-toxic and therapeutically effective in appropriate disease models, the drug may be administered to patients by that route of administration determined to make the drug bio-available, in an appropriate solid or solution formulation, to gain the desired therapeutic benefit.

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human dopamine D_1 receptor, for example with a coding sequence included within the sequence shown in Figure 1. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. Detection of nucleic acid encoding human dopamine D_1 receptors is useful as a diagnostic test for any disease process in which levels of expression of the corresponding dopamine D_1 receptor is altered. DNA probe molecules are produced by insertion of a DNA molecule which encodes human dopamine D_1 receptor or fragments thereof into suitable vectors, such as plasmids or bacteriophages, followed by insertion into itable bacterial host cells and replication and harvesting of the

DNA probes, all using methods well known in the art. For example, the DNA may be extracted from a cell lysate using phenol and ethanol, digested with restriction enzymes corresponding to the insertion sites of the DNA into the vector (discussed above), electrophoresed, and cut out of the resulting gel. Examples of such DNA molecules are shown in Figure 1. The probes are useful for 'in situ' hybridization or in order to locate tissues which express this gene family, or for other hybridization assays for the presence of these genes or their mRNA in various biological tissues. In addition, synthesized oligonucleotides (produced by a DNA synthesizer) complementary to the sequence of a DNA molecule which encodes a human dopamine D_1 receptor of are useful as probes for these genes, for their associated mRNA, or for the isolation of related genes by homology screening of genomic or cDNA libraries, or by the use of amplification techniques such as the polymerase chain reaction.

This invention also provides a method of detecting expression of a dopamine D_1 receptor on the surface of a cell by detecting the presence of mRNA coding for a dopamine D_1 receptor which comprises obtaining total mRNA from the cell using methods well known in the art and contacting the mRNA so obtained with a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human dopamine D_1 receptor under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the dopamine D_1 receptor by the cell. Hybridization of probes to target nucleic acid molecules such as mRNA molecules employs techniques well

known in the art.   In one possible means of performing this
method, nucleic acids are extracted by precipitation from
lysed cells and the mRNA is isolated from the extract using
a column which binds the poly-A tails of the mRNA molecules.

5   The mRNA is then exposed to radioactively labelled probe on
a nitrocellulose membrane, and the probe hybridizes to and
thereby labels complementary mRNA sequences. Binding may be
detected by autoradiography or scintillation counting.
However, other methods for performing these steps are well

10   known to those skilled in the art, and the discussion above
is merely an example.

This invention provides an antisense oligonucleotide having
a sequence capable of binding specifically with any

15   sequences of an mRNA molecule which encodes a human dopamine
D_1 receptor so as to prevent translation of the mRNA
molecule. The antisense oligonucleotide may have a sequence
capable of binding specifically with any sequences of the
DNA molecule whose sequence is shown in Figure 1.   A

20   particular example of an antisense oligonucleotide is an
antisense oligonucleotide comprising chemical analogues of
nucleotides.

This invention also provides a pharmaceutical composition

25   comprising an amount of the oligonucleotide described above
effective to reduce expression of a human dopamine D_1
receptor by passing through a cell membrane and binding
specifically with mRNA encoding a human dopamine D_1 receptor
in the cell so as to prevent its translation and a

30   pharmaceutically acceptable hydrophobic carrier capable of
passing through a cell membrane. The oligonucleotide may be
coupled to a substance which inactivates mRNA, such as a
ribozyme.   The pharmaceutically acceptable hydrophobic

carrier capable of passing through cell membranes may also
comprise a structure which binds to a receptor specific for
a selected cell type and is thereby taken up by cells of the
selected cell type. The structure may be part of a protein

5   known to bind a cell-type specific receptor, for example an
insulin molecule, which would target pancreatic cells. DNA
molecules having coding sequences substantially the same as
the coding sequence shown in Figure 1 may be used as the
oligonucleotides of the pharmaceutical composition.

10

This invention also provides a method of treating
abnormalities which are alleviated by reduction of
expression of a dopamine D_1 receptor which comprises
administering to a subject an amount of the pharmaceutical

15   composition described above effective to reduce expression
of the dopamine D_1 receptor by the subject. This invention
further provides a method of treating an abnormal condition
related to dopamine D_1 receptor activity which comprises
administering to a subject an amount of the pharmaceutical

20   composition described above effective to reduce expression
of the dopamine D_1 receptor by the subject. Several
examples of such abnormal conditions are dementia,
Parkinson's disease, abnormal cognitive functioning such as
schizophrenia, tardive dyskinesia, renal failure, and

25   failure of vascular control, abnormal circadian rhythms, and
abnormal visual activity.

Antisense oligonucleotide drugs inhibit translation of mRNA
encoding these receptors. Synthetic oligonucleotides, or

30   other antisense chemical structures are designed to bind to
mRNA encoding the dopamine D_1 receptor and inhibit
translation of mRNA and are useful as drugs to inhibit
expression of dopamine D_1 receptor genes in patients. This

invention provides a means to therapeutically alter levels
of expression of human dopamine D_1 receptors by the use of
a synthetic antisense oligonucleotide drug (SAOD) which
inhibits translation of mRNA encoding these receptors.
5   Synthetic oligonucleotides, or other antisense chemical
structures designed to recognize and selectively bind to
mRNA, are constructed to be complementary to portions of the
nucleotide sequences shown in Figure 1 of DNA, RNA or of
chemically modified, artificial nucleic acids. The SAOD is
10   designed to be stable in the blood stream for administration
to patients by injection, or in laboratory cell culture
conditions, for administration to cells removed from the
patient. The SAOD is designed to be capable of passing
through cell membranes in order to enter the cytoplasm of
15   the cell by virtue of physical and chemical properties of
the SAOD which render it capable of passing through cell
membranes (e.g. by designing small, hydrophobic SAOD
chemical structures) or by virtue of specific transport
systems in the cell which recognize and transport the SAOD
20   into the cell. In addition, the SAOD can be designed for
administration only to certain selected cell populations by
targeting the SAOD to be recognized by specific cellular
uptake mechanisms which binds and takes up the SAOD only
within certain selected cell populations. For example, the
25   SAOD may be designed to bind to a receptor found only in a
certain cell type, as discussed above. The SAOD is also
designed to recognize and selectively bind to the target
mRNA sequence, which may correspond to a sequence contained
within the sequences shown in Figure 1, by virtue of
30   complementary base pairing to the mRNA. Finally, the SAOD
is designed to inactivate the target mRNA sequence by any of
three mechanisms: 1) by binding to the target mRNA and thus
inducing degradation of the mRNA by intrinsic cellular

mechanisms such as RNAse I digestion, 2) by inhibiting translation of the mRNA target by interfering with the binding of translation-regulating factors or of ribosomes, or 3) by inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups, which either degrade or chemically modify the target mRNA. Synthetic antisense oligonucleotide drugs have been shown to be capable of the properties described above when directed against mRNA targets (J.S. Cohen, Trends in Pharm. Sci. 10, 435 (1989); H.M. Weintraub, Sci. Am. January (1990) p. 40). In addition, coupling of ribozymes to antisense oligonucleotides is a promising strategy for inactivating target mRNA (N. Sarver et al., Science 247, 1222 (1990)). An SAOD serves as an effective therapeutic agent when it is administered to a patient by injection, or when the patient's target cells are removed, treated with the SAOD in the laboratory, and replaced in the patient. In this manner, an SAOD serves as a therapy to reduce receptor expression in particular target cells of a patient, in any clinical condition which may benefit from reduced expression of dopamine D_1 receptor.

This invention provides an antibody directed to the human dopamine D_1 receptor, for example a monoclonal antibody directed to an epitope of a human dopamine D_1 receptor present on the surface of a cell and having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human dopamine D_1 receptor included in the amino acid sequence shown in Figure 1. Amino acid sequences may be analyzed by methods well known in the art to determine whether they produce hydrophobic or hydrophilic regions in the proteins which they build. In the case of cell membrane proteins, hydrophobic regions are

well known to form the part of the protein that is inserted into the lipid bilayer which forms the cell membrane, while hydrophilic regions are located on the cell surface, in an aqueous environment. Therefore antibodies to the hydrophilic amino acid sequences shown in Figure 1 will bind to a surface epitope of a human dopamine D_1 receptor, as described. Antibodies directed to human dopamine D_1 receptor may be serum-derived or monoclonal and are prepared using methods well known in the art. For example, monoclonal antibodies are prepared using hybridoma technology by fusing antibody producing B cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired antibody. Cells such as SR3T3 cells or Ltk- cells may be used as immunogens to raise such an antibody. Alternatively, synthetic peptides may be prepared using commercially available machines and the amino acid sequences shown in Figure 1. As a still further alternative, DNA, such as a cDNA or a fragment thereof, may be cloned and expressed and the resulting polypeptide recovered and used as an immunogen. These antibodies are useful to detect the presence of human dopamine D_1 receptors encoded by the isolated DNA, or to inhibit the function of the receptors in living animals, in humans, or in biological tissues or fluids isolated from animals or humans.

This invention provides a pharmaceutical composition which comprises an amount of an antibody directed to the human dopamine D_1 receptor effective to block binding of naturally occurring ligands to the dopamine D_1 receptor, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to an epitope of a human dopamine D_1 receptor present on the surface of a cell and having an amino acid

sequence substantially the same as an amino acid sequence for a cell surface epitope of the human dopamine D_1 receptor included in the amino acid sequence shown in Figure 1 are useful for this purpose.

This invention also provides a method of treating abnormalities which are alleviated by reduction of expression of a human dopamine D_1 receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to block binding of naturally occurring ligands to the dopamine D_1 receptor and thereby alleviate abnormalities resulting from overexpression of a human dopamine D_1 receptor. Binding of the antibody to the receptor prevents the receptor from functioning, thereby neutralizing the effects of overexpression. The monoclonal antibodies described above are both useful for this purpose. This invention additionally provides a method of treating an abnormal condition related to an excess of dopamine D_1 receptor activity which comprises administering to a subject an amount of the pharmaceutical composition described above effective to block binding of naturally occurring ligands to the dopamine D_1 receptor and thereby alleviate the abnormal condition. Several examples of such abnormal conditions are dementia, Parkinson's disease, abnormal cognitive functioning such as schizophrenia, tardive dyskinesia, renal failure, and failure of vascular control, abnormal circadian rhythms, and abnormal visual activity.

This invention provides a method of detecting the presence of a human dopamine D_1 receptor on the surface of a cell which comprises contacting the cell with an antibody directed to the human dopamine D_1 receptor, under conditions

permitting binding of the antibody to the receptor,
detecting the presence of the antibody bound to the cell,
and thereby the presence of the human dopamine D_1 receptor
on the surface of the cell. Such a method is useful for
determining whether a given cell is defective in expression
of dopamine D_1 receptors on the surface of the cell. Bound
antibodies are detected by methods well known in the art,
for example by binding fluorescent markers to the antibodies
and examining the cell sample under a fluorescence
microscope to detect fluorescence on a cell indicative of
antibody binding. The monoclonal antibodies described above
are useful for this purpose.

This invention provides a transgenic nonhuman mammal
expressing DNA encoding a human dopamine D_1 receptor. This
invention also provides a transgenic nonhuman mammal
expressing DNA encoding a human dopamine D_1 receptor so
mutated as to be incapable of normal receptor activity, and
not expressing native dopamine D_1 receptor. This invention
also provides a transgenic nonhuman mammal whose genome
comprises antisense DNA complementary to DNA encoding a
human dopamine D_1 receptor so placed as to be transcribed
into antisense mRNA which is complementary to mRNA encoding
a dopamine D_1 receptor and which hybridizes to mRNA encoding
a dopamine D_1 receptor thereby reducing its translation.
The DNA may additionally comprise an inducible promoter or
additionally comprise tissue specific regulatory elements,
so that expression can be induced, or restricted to specific
cell types. Examples of DNA are DNA or cDNA molecules
having a coding sequence substantially the same as the
coding sequence shown in Figure 1. An example of a
transgenic animal is a transgenic mouse. Examples of tissue
specificity-determining regions are the metallothionein

promotor (Low, M.J., Lechan, R.M., Hammer, R.E. et al. Science 231:1002-1004 (1986)) and the L7 promotor (Oberdick, J., Smeyne, R.J., Mann, J.R., Jackson, S. and Morgan, J.I. Science 248:223-226 (1990)).

Animal model systems which elucidate the physiological and behavioral roles of human dopamine D_1 receptors are produced by creating transgenic animals in which the expression of a dopamine D_1 receptor is either increased or decreased, or the amino acid sequence of the expressed dopamine D_1 receptor protein is altered, by a variety of techniques. Examples of these techniques include: 1) Insertion of normal or mutant versions of DNA encoding a human dopamine D_1 receptor or homologous animal versions of these genes, by microinjection, retroviral infection or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal (Hogan B. et al. Manipulating the Mouse Embryo, A Laboratory Manual, Cold Spring Harbor Laboratory (1986)); 2) Homologous recombination (Capecchi M.R. Science 244:1288- 1292 (1989); Zimmer, A. and Gruss, P. Nature 338:150-153 (1989)) of mutant or normal, human or animal versions of the gene with the native gene locus in transgenic animals to alter the regulation of expression or the structure of the dopamine D_1 receptor. The technique of homologous recombination is well known in the art. This technique replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native receptor but does express, for example, an inserted mutant receptor, which has replaced the native receptor in the animal's genome by recombination, resulting in underexpression of the receptor (in more detail, mutually homologous regions of the insert DNA and genomic DNA pair

with each other, resulting in the replacement of the homologous regions of genomic DNA and regions between the homologous regions with the insert). Microinjection adds genes to the genome, but does not remove them, and so is useful for producing an animal which expresses its own and added receptors, resulting in overexpression of the receptor. One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as M2 medium (Hogan B. et al. Manipulating the Mouse Embryo, A Laboratory Manual, Cold Spring Harbor Laboratory (1986)). DNA or cDNA encoding a human dopamine D_1 receptor is purified from a vector (such as plasmid pdopD1-GL-30 described above) by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the trans-gene. Alternatively or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg cell, and is used here only as an example.

-34-

Since the normal action of receptor-specific drugs is to
activate or to inhibit the receptor, the transgenic animal
model systems described above are useful for testing the
biological activity of potential drugs directed against the
dopamine D_1 receptor even before such drugs become
available.   These animal model systems are useful for
predicting or evaluating possible therapeutic applications
of drugs which activate or inhibit the dopamine D_1 receptor
by inducing or inhibiting expression of the native or trans-
gene and thus increasing or decreasing expression of normal
or mutant dopamine D_1 receptors in the living animal. Thus,
a model system is produced in which the biological activity
of a potential drug directed against the dopamine D_1
receptor can be evaluated before the actual development of
such a drug.   The transgenic animals which over or under
produce the dopamine D_1 receptor indicate by their
physiological state whether over or under production of the
dopamine D_1 receptor is therapeutically useful.   The
transgenic model system is therefore useful to evaluate
potential drug action. For example, it is well known in the
art that a drug such as an antidepressant acts by blocking
neurotransmitter uptake, and thereby increases the amount of
neurotransmitter in the synaptic cleft.   The physiological
result of this action is to stimulate reduced production of
receptor by the affected cells, leading eventually to
underexpression.   Therefore, an animal which is engineered
to underexpress receptor is useful as a test system to
investigate whether the action of a drug which results in
underexpression is in fact therapeutic. Again, for example,
if overexpression is found to lead to abnormalities, then a
drug which can down-regulate or act as an antagonist to
dopamine D_1 receptor is indicated as worth developing. If
a promising therapeutic application is uncovered by these

animal model systems, activation or inhibition of the
dopamine D_1 receptor can be achieved therapeutically either
by producing agonist or antagonist drugs directed against
the dopamine D_1 receptor, or indeed by any method which
5   increases or decreases the expression of the dopamine D1
receptor.

This invention provides a method of determining the
physiological effects of expressing varying levels of human
10   dopamine D_1 receptors which comprises producing a transgenic
nonhuman animal whose levels of human dopamine D_1 receptor
expression are varied by use of an inducible promoter which
regulates human dopamine D_1 receptor expression. This
invention also provides a method of determining the
15   physiological effects of expressing varying levels of human
dopamine D_1 receptors which comprises producing a panel of
transgenic nonhuman animals each expressing a different
amount of human dopamine D_1 receptor. Such animals may be
produced by introducing different amounts of DNA encoding a
20   human dopamine D_1 receptor into the oocytes from which the
transgenic animals are developed.

This invention also provides a method for identifying a
substance capable of alleviating abnormalities resulting
25   from overexpression of a human dopamine D_1 receptor
comprising administering the substance to a transgenic
nonhuman mammal expressing at least one artificially
introduced DNA molecule encoding a human dopamine D_1
receptor and determining whether the substance alleviates
30   the physical and behavioral abnormalities displayed by the
transgenic nonhuman mammal as a result of overexpression of
a human dopamine D_1 receptor. Examples of DNA molecules are
DNA or cDNA molecules having a coding sequence substantially

the same as the coding sequence shown in Figure 1.

This invention provides a pharmaceutical composition
comprising an amount of the substance described <u>supra</u>
5   effective to alleviate the abnormalities resulting from
overexpression of dopamine D_1 receptor and a
pharmaceutically acceptable carrier.

This invention further provides a method for treating the
10   abnormalities resulting from overexpression of a human
dopamine D_1 receptor which comprises administering to a
subject an amount of the pharmaceutical composition
described above effective to alleviate the abnormalities
resulting from overexpression of a human dopamine D_1
15   receptor.

This invention provides a method for identifying a substance
capable of alleviating the abnormalities resulting from
underexpression of a human dopamine D_1 receptor comprising
20   administering the substance to the transgenic nonhuman
mammal described above which expresses only nonfunctional
human dopamine D_1 receptor and determining whether the
substance alleviates the physical and behavioral
abnormalities displayed by the transgenic nonhuman mammal as
25   a result of underexpression of a human dopamine D_1 receptor.

This invention also provides a pharmaceutical composition
comprising an amount of a substance effective to alleviate
abnormalities resulting from underexpression of dopamine D_1
30   receptor and a pharmaceutically acceptable carrier.

This invention further provides a method for treating the
abnormalities resulting from underexpression of a human

dopamine D_1 receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from underexpression of a human dopamine D_1

5   receptor.

This invention provides a method for diagnosing in a subject a predisposition to a disorder associated with the expression of a specific human dopamine D_1 receptor allele

10   which comprises: a. isolating DNA from victims of the disorder, b. digesting the isolated DNA of step a with at least one restriction enzyme, c. electrophoretically separating the resulting DNA fragments on a sizing gel, d. contacting the resulting gel with a nucleic acid probe

15   capable of specifically hybridizing to DNA encoding a human dopamine D_1 receptor and labelled with a detectable marker, e. detecting labelled bands which have hybridized to the DNA encoding a human dopamine D_1 receptor labelled with a detectable marker to create a band pattern specific to the

20   DNA of victims of the disorder, f. preparing the subject's DNA by steps a-e to produce detectable labeled bands on a gel, and g. comparing the band pattern specific to the DNA of victims of the disorder of step e and the subject's DNA of step f to determine whether the patterns are the same or

25   different and thereby to diagnose predisposition to the disorder if the patterns are the same. This method may also be used to diagnose a disorder associated with the expression of a specific human dopamine D_1 receptor allele. This method makes use of restriction fragment length

30   polymorphisms in the gene of interest, which may itself encode an abnormal phenotype, or may encode or predispose to an abnormal phenotype in one of its allelic forms, or may encode an abnormal phenotype when present in mutant form.

A DNA probe is a useful genetic probe for an allelic abnormality. An allele of a gene will have a specific restriction fragment pattern when its isolated DNA is digested with a single restriction enzyme or panel of restriction enzymes, because of polymorphisms in the areas of the gene which have nucleotide sequences that form sites for restriction enzymes. For example, the gene may have the sequence AATTC which forms the site for the enzyme EcoRI. Its allele may have in the same area the sequence AAATC. When the isolated DNA comprising the gene and its allele are digested with EcoRI by methods well known in the art, the gene will be cut at the site described and this cut will create a fragment of a length determined by the location of the next EcoRI site (assuming this is a single-enzyme digest). The allele will not be cut at this site, therefore the fragment generated by the digest will be longer. When the DNA digest is run on an agarose or polyacrylamide sizing gel and hybridized with the detectably labelled DNA probe for the gene, the detectable band visualized on the gel will correspond to the length of the restriction fragments produced. If the fragment is the "long" fragment, then this result indicates that the allele is carried by the DNA digested. If the presence of the allelic form of the gene is associated with a predisposition to a phenotypic abnormality, then the predictive power of such an analysis is important. If the abnormality already exists, then this test is useful for diagnosis and differential diagnosis. An allele is given only as an example. This method may be used to detect mutations and polymorphisms of a gene of interest, or the gene itself. Methods for isolating DNA (from a source such as a blood or tissue sample, for example) are well known in the art. Methods of visualizing a labeled nucleic acid probe hybridized to a gel are also

well known in the art. For example, the DNA on a gel is
denatured with base, incubated with a radioactively labeled
probe, and a filter (usually nitrocellulose) is placed over
the gel, transferring the fragments on the gel to the
5   filter. A piece of film is laid over the filter. The
fragments which have hybridized to the probe will expose the
film and leave a band marking their positions in the gel.

This invention provides a method of preparing the isolated
10   dopamine D_1 receptor which comprises inducing cells to
express dopamine D_1 receptor, recovering the receptor from
the resulting cells, and purifying the receptor so
recovered. An example of an isolated dopamine D_1 receptor
is an isolated protein having substantially the same amino
15   acid sequence as the amino acid sequence shown in Figure 1.
For example, cells can be induced to express receptors by
exposure to substances such as hormones. The cells can then
be homogenized and the receptor isolated from the homogenate
using an affinity column comprising, for example, dopamine,
20   or antibody to the dopamine D_1 receptor, or another
substance which is known to bind to the receptor. The
resulting fractions can then be purified by contacting them
with an ion exchange column, and determining which fraction
contains receptor activity or binds anti-receptor
25   antibodies. These methods are provided as examples, and do
not exclude the use of other methods known in the art for
isolating proteins.

This invention provides a method of preparing the isolated
30   dopamine D_1 receptor which comprises inserting nucleic acid
encoding dopamine D_1 receptor in a suitable vector,
inserting the resulting vector in a suitable host cell,
recovering the receptor produced by the resulting cell, and

purifying the receptor so recovered. An example of an
isolated dopamine D_1 receptor is an isolated protein having
substantially the same amino acid sequence as the amino acid
sequence shown in Figure 1. This method for preparing

5   dopamine D_1 receptor uses recombinant DNA technology methods
well known in the art. For example, isolated nucleic acid
encoding dopamine D_1 receptor is inserted in a suitable
vector, such as an expression vector. A suitable host
cell, such as a bacterial cell, or a eucaryotic cell such as

10   a yeast cell, is transfected with the vector. The dopamine
D_1 receptor is isolated from the culture medium by affinity
purification or by chromatography or by other methods well
known in the art.

15   Applicants have identified individual receptor subtype
proteins and have described methods for the identification
of pharmacological compounds for therapeutic treatments.
Pharmacological compounds which are directed against
specific receptor subtypes provide effective new therapies

20   with minimal side effects.

Disturbances of dopaminergic neurotransmission have been
associated with a wide range of neurological, endocrine, and
psychiatric disorders, including Parkinson's disease,

25   tardive dyskinesia, and schizophrenia. The neuroleptics,
which have highest affinity for D_2 receptors have major side
effects involving movement disorders and hypersecretion of
prolactin. Drugs used in the treatment of Parkinson's
disease cause nausea, vomiting, choreiform movements,

30   psychiatric disturbances including hallucinations, and
cardiovascular disorders. Some of these effects are likely
to be due to actions on D_1 receptors or to a disruption in
the balance of activity between the D_1 and D_2 systems

(Abbott, A., 1990; TIPS 11: 49-51). In fact some therapeutic benefit of D$_1$ antagonists which lack D$_2$ activity may be obtained. (Hess, E.J. and Creese, I., in Neurobiology of Central D$_1$ Receptors, eds, G.R. Breese and I. Creese pp. 53-72) Drugs selectively targeted to D$_1$ receptor may be useful neuroleptics without resulting in the tardive dyskinesia thought to be the result of D$_2$ receptor up-regulation caused by chronic D$_2$ antagonism (Hess, E.J. and Creese, I., in Neurobiology of Central D$_1$ Receptors, eds, G.R. Breese and I. Creese pp. 53-72). Furthermore, evidence provided by the anatomical distribution of D$_1$ receptors in the brain suggest roles for D$_1$ selective drugs in cognitive function, control of visual activity and circadian rhythms (Dawson, T., Gelhert, D., McCabe, R., Barnett, A. , and Wamsley, J. 1986; J. Neurosci. 6:2352-2365). Finally, the distribution of D$_1$ receptors on the renal vasculature indicates potential therapeutic value of selective D$_1$ agents to ameliorate renal failure secondary to heart attack (Missale, C., Castelleti, L., Memo, M., Carruba, M., and Spano, P. 1988; J. Cardiovascular Research 11: 643-650.) Its general action on vascular smooth muscle in other portions of the vascular tree may indicate a general role in cardiovascular control (Missale, as above; Hilditch, A. and Drew, G.M. 1985, TIPS 6:396-400).

In animal models, D$_1$-selective benzazepines induce intense grooming (Molloy, A.G. and Waddington, J.L. (1987), Psychopharmacology (Berlin) 92, 164-168), inhibit spontaneous locomotion (Hjorth, S. and Carlsson, A. (1988), J. Neural Transm. 72, 83-97), and generally seem to facilitate D2 receptor activities (Waddington, J.L. (1986), Biochem. Pharmacol. 35, 3661-3667; Hjorth, S. and Carlsson, A. (1988), J. Neural Transm. 72, 83-97). These actions

produce therapeutic applications in enhancing Parkinson's
disease or antipsychotic therapies with existing D_2
antagonists (Waddington, J.L. (1986), Biochem. Pharmacol.
35, 3661-3667). In human peripheral arteries, D_{A1} receptors

5   mediate vasodilation (Toda, N., Okunishi, H., and Okamura,
T. (1989), Arch Int. Pharmacodyn. Ther. 297, 86-97).
Clinical trials with the selective D_{A1} receptor agonist,
fenoldopam, have revealed a potent renal vasodilatory action
that could provide an attractive alternative therapy for

10   treating hypertensive and congestive heart failure patients
(Carey, R.A. and Jacob, L. (1989), J. Clin Pharmacol. 29,
207-211). Development of more selective D_1 agonists and
antagonists will expand existing D_1 therapeutic applications
and suggest new ones.

15
This invention identifies for the first time a new human
receptor protein, the dopamine D_1 receptor, its amino acid
sequences, and its human gene, clone GL-30. The first
isolated human cDNA and genomic clone encoding dopamine D_1

20   receptor are identified and characterized herein. The
information and experimental tools provided by this
discovery will be useful to generate new therapeutic agents,
and new therapeutic or diagnostic assays for the new
receptor protein, associated mRNA molecules, or associated

25   genomic DNA.

The invention will be better understood by reference to the
Experimental Details which follow, but those skilled in the
art will readily appreciate that the specific experiments

30   detailed are only illustrative of the invention as described
more fully in the claims which follow thereafter.

-43-

Experimental Details:

Homology Cloning. A human spleen genomic library, provided
by Dr. Jeffrey V. Ravetch (Sloan-Kettering Institute, New
York, NY), was screened using the 1.6-kilobase (kb) XbaI-
BamHI fragment from the human 5-hydroxytryptamine (5-HT$_{1A}$)
receptor gene as a probe. The probe was labeled with ^{32}P by
the method of random priming. Hybridization was performed
at 40°C in a solution containing 25% formamide, 10% dextran
sulfate, 5X SSC (1X SSC is 0.15 M sodium chloride, 0.015 M
sodium citrate) 1X Denhardt's (0.02% polyvinyl-pyrrolidone,
0.02% Ficoll, and 0.02% bovine serum albumin), and 200 μg/ml
of sonicated salmon sperm DNA. The filters were washed at
40°C in 0.1X SSC containing 0.1% sodium-dodecyl-sulfate
(SDS) and exposed at -70°C to Kodak XAR film in the presence
of an intensifying screen. Lambda phage hybridizing to the
probe were plaque purified and DNA was prepared for Southern
blot analysis (Maniatis et al., Molecular Cloning, Cold
Spring Harbor, 1982; E. Southern, J. Mol. Biol. 98:503,
1975). For subcloning and further southern blot analysis
DNA was inserted into pUC18 (Pharmacia, Piscataway, N.J.).

DNA Sequencing
Nucleotide sequence analysis was done by the Sanger dideoxy
nucleotide chain-termination method (S. Sanger, et al.,
Proc. Natl. Acad. Sci., 74: 5463-5467, 1977) or denatured
double-stranded plasmid templates (Chen and Seeb...g, DNA 4:
165, 1985) using Sequenase (U.S. Biochemical Corp.,
Cleveland, Ohio).

Receptor Expression in Transfected Mammalian Cells
To confirm the functional identity of the newly isolated
gene clone GL-30 was expressed in cultured cell lines. The

entire coding region of GL-30, including 113 base pairs of
5' untranslated sequence, and approximately 1.3 kb of 3'
untranslated sequence, was cloned into the eucaryotic
expression vector pcEXV-3 (Miller, J. and Germain, R.N.
(1986), J. Exp. Med. 164: 1478-89). The resulting plasmid
was transiently transfected into Cos-7 cells using the DEAE-
dextran procedure (Cullen, Methods in Enz., 152: 684-704,
1987).

Measurement of cAMP Formation
The transiently transfected plates were incubated in
Dulbecco's modified Eagle's medium (DMEM, Specialty Media,
Lavallette, NJ), 5mM theophylline, 10mM Hepes, 10μM
pargyline, 10μM propanolol, and/or 10μM SCH-23390 for 20
minutes at 37°C, 5% CO_2. In these experiments, the β-
adrenergic antagonist propanolol was included in the assay
to preclude stimulation of the endogenous Cos-7 cell β-
adrenergic receptor by dopamine. Dopamine or SKF-38393 was
then added to a final concentration of 1μM and incubated for
an additional 10 minutes at 37°C, 5% CO_2. The media was
aspirated and the reaction stopped by the addition of 100mM
HCl. The plates were stored at 4°C for 15 minutes,
centrifuged for 5 minutes, 500 x g to pellet cellular
debris, and the supernatant aliquotted and stored at -20°C
prior to assessment of cAMP formation by radioimmunoassay
(cAMP Radioimmunoassay Kit, Advanced Magnetics, Cambridge,
MA).

Membrane Preparation
Membranes were harvested from transfected Cos-7 cells which
were grown to 100% confluency. The cells were washed twice
with phosphate-buffered saline (PBS), scraped into 5 ml of
ice-cold PBS and centrifuged at 200 x g for 5 minutes at

4°C. The pellet was resuspended in 2.5 ml ice-cold Tris buffer (20mM Tris HCl, pH 7.4 at 23°C, 5mM EDTA), hand homogenized in a Wheaton tissue grinder and the lysate centrifuged at 200 x g for 5 minutes at 4°C to pellet large fragments. The supernatant was then centrifuged at 40,000 x g for 20 minutes at 4°C. The membranes were washed once and resuspended in the homogenization buffer. All preparations were kept on ice and assays were run on the day on which the membranes were collected. Protein concentration was determined by the method of Bradford (Anal. Biochem. 72: 248-54 (1976)) using bovine serum albumin as standard.

Radioligand Binding Studies

Binding assays were performed in triplicate in total volume of 250 μl containing buffer (50mM Tris HCl, 10mM $MgSO_4$, 1.5mM EDTA, 150mM NaCl, 0.1% ascorbate, 10 μM pargyline, pH 7.4 at 4°C), [^{3}H]SCH-23390 (87 Ci/mmol; DuPont-NEN, Wilmington, DE) and tested drugs. In competition binding experiments, 0.5-0.6 nM [^{3}H]SCH-23390 was inhibited by various concentrations of unlabeled drugs. Binding was initiated by the addition of membrane preparation (10-20 μg protein) and carried out at 22°C for 90 minutes. Specific binding was 95% of total binding at 0.5 nM [^{3}H]SCH-23390. For saturation experiments, membranes were incubated with [^{3}H]SCH-23390 over the concentration range of 0.01-6.5 nM. Incubations were allowed to proceed for 150 minutes at 22°C to ensure that equilibrium was achieved at the lowest concentrations of radioligand. Nonspecific binding was defined in the presence of 10μM (+) butaclamol. The reaction was terminated by rapid filtration through Whatman GF/B glass fiber filters (presoaked with 0.5% polyethyleneamine, pH 7.4), using a Brandel 48R cell

harvester (Brandel; Gaithersburg, MD). Filters were washed
for 5 seconds with iced buffer to reduce nonspecific
binding. Dried filters were transferred to scintillation
vials and radioactivity was determined by liquid
scintillation counting (Beckman LS 1701; Beckman
Instruments, Fullerton, CA). Ready Safe (Beckman) was used
as the scintillant and the counting efficiency was 50%.
Analysis of saturation and competition data were performed
by computer-assisted nonlinear regression (DeLean et al.,
1978; programs Accucomp and Accufit; Lundon Software,
Chagrin Falls, OH). IC_{50} values were converted to K_i values
by the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

Experimental Results:

Isolation of a genomic clone encoding a dopamine D_1
receptor.
We have screened human genomic spleen and human genomic
placental libraries with the 1.6 kb Xba-1-Bam-H1 restriction
fragment derived from the gene for the $5-HT_{1A}$ receptor. A
total of 59 clones were isolated and were characterized by
restriction endonuclease mapping. One clone (designated GL-
30) was isolated as an approximately 4.0 kb EcoRI-Bgl-II
fragment was subcloned into pUC-18 and subject to sequence
analysis.

Predicted Structure of the receptor encoded by GL-30
DNA sequence information obtained from GL-30 is shown in
Figure 1. An open reading frame encoding a protein of 477
amino acids in length, having a relative molecular mass
(M_r) of approximately 53kD. A comparison of this protein
sequence with previously characterized neurotransmitter
receptors indicates that clone GL-30 is a new member of a

family of molecules which span the lipid bilayer seven times and couple to guanine nucleotide regulatory proteins (the G protein-coupled receptor family). A variety of structural features which are invariant in the G-protein coupled receptor family, including the aspartic acid residues of transmembrane regions II and III, the DRY sequence at the end of transmembrane region III, and the conserved proline residues of transmembrane regions IV, V, VI and VII were present in clone GL-30. Both the amino terminus and the extracellular loop 2 (located between transmembrane domains IV and V) of GL-30, contain consensus sites for N-linked glycosylation. In addition, this extracellular loop contains 45 amino acids (as compared to 31 amino acids in the comparable region of the dopamine D_1 receptor) and represents the longest extracellular loop 2 of all the known G-protein coupled receptors. While the carboxy-terminal tails of the dopamine D_1 receptor and GL-30 are approximately the same size, their amino acid sequences are only 41% identical. When compared to all the known G protein-coupled receptors, the greatest homology was found to be with the dopamine D_1 receptor. While the overall homology between GL-30 and the human dopamine D_1 receptor was 62%, the homology within the seven membrane spanning domains was 83% (Figure 2).

Discussion

Applicants have cloned and characterized a DNA molecule encoding a new dopamine D_1 receptor by low stringency hybridization to the serotonin 5-HT$_{1A}$ receptor. Although the amino acid sequence homology of clone GL-30 to the 5-HT$_{1A}$ receptor was relatively low (47% transmembrane region identity), comparison of this sequence to previously cloned

-48-

dopamine receptors showed that the closest relationship was to the human dopamine D_1 receptor (83% identity in the transmembrane domains).   In contrast, the transmembrane homology to either the dopamine D_2 or dopamine D_3 receptors

5   was only 53% and 48%, respectively.

Clone GL-30 was expressed in Cos-7 cells in order to characterize the pharmacological binding properties of the expressed receptor protein.   [^{3}H]SCH-23390, a highly

10   selective D_1 antagonist in the rat (Billard, W. et al. (1984) Life Sci. 35: 1885-93), non-human primate (Madras, B.K. et al. (1988) J. Neurochem. 51: 934-43) and human brain (DeKeyser, J. et al. (1988) Brain Res. 443: 77-84; Raisman, R. et al. (1985) Eur. J. Pharmacol. 113: 467-68) binds to

15   this receptor with an apparent dissociation constant (K_d) of 0.65 nM, in good agreement with values reported for mammalian brain homogenates (Billard et al. (1984) supra; DeKeyser et al. (1988) supra; Raisman et al. (1985) supra; Reader, T.A. et al. (1989) Naunyn-Schmiedeberg's Arch.

20   Pharmacol. 340: 617-25).   This dissociation constant is nearly identical to that previously reported for the cloned D_1 receptor expressed in Cos-7 cells, K_d=0.3-0.6 nM, (Dearry et al. (1990) supra; Sunahara et al. (1990) supra; Zhou et al. (1990) supra).

25

Pharmacological characterization of the GL-30 clone showed binding of [^{3}H]SCH-23390 to a site which clearly exhibited a D_1-like pharmacology (Table 1).   The rank order of potencies of dopaminergic antagonists in displacing the

30   binding shows that the most potent compounds are those previously identified as having the highest affinity for the D_1 site (e.g. SCH-23390, cis-flupenthixol and (+) butaclamol).   Among other drugs classified as dopamine

receptor antagonists, bulbocapnine, haloperidol and clozapine yielded K_i values comparable to those reported for the D_1 receptor in native rat and human brain tissues, and for Cos-7 cells transiently transfected with the previously

5   cloned D_1 gene. The largest difference found between the affinities of antagonists for this newly cloned receptor and those reported for the previously cloned D_1 receptor was for (+) butaclamol which was 6-18 fold less potent at the dopamine $D_{1\beta}$ receptor. Antagonist competition curves were

10   of uniformly steep slope ($n_H \approx 1.0$) suggesting the presence of a single D_1 dopamine receptor. The low affinity of (-) sulpiride and quinpirole to displace $[^3H]$SCH-23390 binding is congruent with the D_2 selectivity of such drugs. The biogenic amine neurotransmitters serotonin and

15   norepinephrine were inactive in inhibiting the binding of the antagonist radioligand.

In contrast to the data on antagonist binding, the rank order of potencies and apparent dissociation constants

20   obtained for dopaminergic agonists did not display a high degree of correlation with those found in native brain tissues, in peripheral preparations, or in the previously characterized D_1 receptor clone. Dopamine displaced $[^3H]$SCH-23390 binding with ≈ 10-20 fold higher affinity

25   (K_i=159 nM) than that reported for D_1 receptors in either the brain or in the periphery under the assay condition used. The competition curve for dopamine in these experiments had a relatively shallow slope, indicating the existence of both high and low affinity binding components.

30   The assay conditions were chosen to match those used in assays of the previously cloned D_1 receptor, and are expected to promote the low affinity configuration of the receptor. Although this dopamine $D_{1\beta}$ receptor has

pharmacological and functional properties similar to D_1 receptors previously characterized in the brain and the periphery, its agonist profile makes it a unique receptor.

Cos-7 cells transfected with clone GL-30 exhibited dopamine stimulated cAMP production at a level 13 fold above the basal rate. This effect of dopamine was blocked by the D_1 selective antagonist SCH-23390. The D_1 selective partial agonist SKF-38393 stimulated cAMP accumulation to a lesser extent than dopamine itself, consistent with its role as a partial agonist (Andersen et al. (1987) _supra_). The previously cloned D_1 receptor was also shown to be coupled to stimulation of adenylate cyclase activity. The observation that the two different D_1 receptor genes encode proteins which functionally couple to the same second messenger pathway reinforces the close relationship shown in their amino acid sequences and pharmacological binding profiles. The existence of two separate genes with similar pharmacology and second messenger coupling suggests that their physiological roles may differ in some other aspect, such as tissue or cell-type distribution, synaptic localization (postsynaptic v. presynaptic autoreceptor), or developmental regulation.

Using gene specific primers for PCR amplification of RNA, the distribution of messenger RNA encoding the dopamine $D_{1\beta}$ receptor was examined. The dopamine $D_{1\beta}$ receptor was found to be widely distributed in a variety of higher brain centers, including brainstem, choroid plexus and hippocampus, suggesting a diverse role in regulating brain functions.

Clone GL-30 is an example of a G protein-coupled receptor

whose entire coding region is contained within a single exon, similar to the dopamine D_1 receptor (Dearry et al. (1990) *supra*; Monsma et al. (1990) *supra*; Sunahara et al. (1990) *supra*; Zhou et al. (1990) *supra*) and many other

5   members of this superfamily.   In contrast, the coding regions of the dopamine D_2 and D_3 receptors are interrupted by several introns (Bunzow et al., 1988; Sokoloff et al., 1990).   Other subfamilies of G protein-coupled receptors (e.g. α_1 or α_2 adrenergic receptors), which consist of

10   closely related subtypes, also share an intron-containing (α_1) or intronless nature (α_2) (Regan and Cotecchia, in press).   Based upon this similarity in intron-exon organization, as well as the close amino acid homology to the previously cloned D_1 receptor, pharmacological binding

15   properties, and second messenger coupling, clone GL-30 can best be characterized as a dopamine D_1 receptor.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: Weinshank, Richard L.
                   Hartig, Paul R.

    (ii) TITLE OF INVENTION: DNA Encoding A Human Dopamine D1
            Receptor And Uses Thereof

   (iii) NUMBER OF SEQUENCES: 5

    (iv) CORRESPONDENCE ADDRESS:
        (A) ADDRESSEE: Cooper & Dunham
        (B) STREET: 30 Rockefeller Plaza
        (C) CITY: New York
        (D) STATE: New York
        (E) COUNTRY: U.S.A.
        (F) ZIP: 10112

    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

    (vi) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER:
        (B) FILING DATE: 10-JUL-1991
        (C) CLASSIFICATION:

    (vii) PRIOR APPLICATION DATA:
        (A) APPLICATION NUMBER: US 551,448
        (B) FILING DATE: 10-JUL-1990

    (viii) ATTORNEY/AGENT INFORMATION:
        (A) NAME: White, John P.
        (B) REGISTRATION NUMBER: 28,678
        (C) REFERENCE/DOCKET NUMBER: 37526-A-PCT

    (ix) TELECOMMUNICATION INFORMATION:
        (A) TELEPHONE: (212) 977-9550
        (B) TELEFAX: (212) 664-0525
        (C) TELEX: 422523 COOP UI

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1771 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

SUBSTITUTE SHEET

        (ii) MOLECULE TYPE: cDNA

        (iii) HYPOTHETICAL: NO

        (iv) ANTI-SENSE: NO

        (vii) IMMEDIATE SOURCE:
              (B) CLONE: GL-30

        (ix) FEATURE:
              (A) NAME/KEY: CDS
              (B) LOCATION: 141..1631

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GCAGCTCATG GTGACCCCCC TCTGGGCTCG AGGGTCCCTT GGCTGAGGGG GCGCATCCTC          60

GGGGTGCCGA TGGGGCTGCC TGGGGGTCGC AGGGCTGAAG TTGGGACCGC GCACAGACCG         120

CCCCTGCAGT CCAGCCCAAA TGC TGC CGC CAG GCA GCA ACG GCA CCG CGT            170
                         Cys Cys Arg Gln Ala Ala Thr Ala Pro Arg
                          1               5                  10

ACC CGG GGC AGT TCG CTC TAT ACC AGC AGC TGG CGC AGG GGA ACG CCG          218
Thr Arg Gly Ser Ser Leu Tyr Thr Ser Ser Trp Arg Arg Gly Thr Pro
                 15              20                  25

TGG GGG GCT CGG CGG GGG CAC CGC CAC TGG GGC CCT CAC AGG TGG TCA          266
Trp Gly Ala Arg Arg Gly His Arg His Trp Gly Pro His Arg Trp Ser
             30                  35                  40

CCG CCT GCC TGC TGA CCC TAC TCA TCA TCT GGA CCC TGC TGG GCA ACG          314
Pro Pro Ala Cys  *  Pro Tyr Ser Ser Ser Gly Pro Cys Trp Ala Thr
             45                  50                  55

TGC TGG TGT GCG CAG CCA TCG TGC GGA GCC GCC ACC TGC GCG CCA ACA          362
Cys Trp Cys Ala Gln Pro Ser Cys Gly Ala Ala Thr Cys Ala Pro Thr
         60                  65                  70

TGA CCA ACG TCT TCA TCG TGT CTC TGG CCG TGT CAG ACC TTT TCG TGG          410
 *  Pro Thr Ser Ser Ser Cys Leu Trp Pro Cys Gln Thr Phe Ser Trp
75                  80                  85                  90

CGC TGC TGG TCA TGC CCT GGA AGG CAG TCG CCG AGG TGG CCG GTT ACT          458
Arg Cys Trp Ser Cys Pro Gly Arg Gln Ser Pro Arg Trp Pro Val Thr
                 95                 100                 105

GGG CCT TTG GAG CGT TCT GCG ACG TCT GGG TGG CCT TCG ACA TCA TGT          506
Gly Pro Leu Glu Arg Ser Ala Thr Ser Gly Trp Pro Ser Thr Ser Cys
             110                 115                 120

GCT CCA CTG CCT CCA TCC TGA ACC TGT GCG TCA TCA GCG TGG ACC GCT          554
```

-54-

```
       Ala Pro Leu Pro Pro Ser  *  Thr Cys Ala Ser Ser Ala Trp Thr Ala
           125              130               135

  5    ACT GGG CCA TCT CCA GGC CCT TCC GCT ACA AGC GCA AGA TGA CTC AGC    602
       Thr Gly Pro Ser Pro Gly Pro Ser Ala Thr Ser Ala Arg  *  Leu Ser
           140              145               150

       GCA TGG CCT TGG TCA TGG TCG GCC TGG CAT GGA CCT TGT CCA TCC TCA    650
       Ala Trp Pro Trp Ser Trp Ser Ala Trp His Gly Pro Cys Pro Ser Ser
           155              160               165               170

 10    TCT CCT TCA TTC CGG TCC AGC TCA ACT GGC ACA GGG ACC AGG CGG CCT    698
       Ser Pro Ser Phe Arg Ser Ser Ser Thr Gly Thr Gly Thr Arg Arg Pro
                   175              180               185

       CTT GGG GCG GGC TGG ACC TGC CAA ACA ACC TGG CCA ACT GGA CGC CCT    746
       Leu Gly Ala Gly Trp Thr Cys Gln Thr Thr Trp Pro Thr Gly Arg Pro
           190              195               200

 15    GGG AGG AGG ACT TTT GGG AGC CCG ACG TGA ATG CAG AGA ACT GTG ACT    794
       Gly Arg Arg Thr Phe Gly Ser Pro Thr  *  Met Gln Arg Thr Val Thr
           205              210               215

       CCA GCC TGA ATC GAA CCT ACG CCA TCT CTT CCT CGC TCA TCA GCT TCT    842
       Pro Ala  *  Ile Glu Pro Thr Pro Ser Leu Pro Arg Ser Ser Ala Ser
           220              225               230

 20    ACA TCC CCG TTG CCA TCA TGA TCG TGA CCT ACA CGC GCA TCT ACC GCA    890
       Thr Ser Pro Leu Pro Ser  *  Ser  *  Pro Thr Arg Ala Ser Thr Ala
       235              240               245               250

       TCG CCC AGG TGC AGA TCC GCA GGA TTT CCT CCC TGG AGA GGG CCG CAG    938
       Ser Pro Arg Cys Arg Ser Ala Gly Phe Pro Pro Trp Arg Gly Pro Gln
                   255              260               265

 25    AGC ACG CGC AGA GCT GCC GGA GCA GCG CAG CCT GCG CGC CCG ACA CCA    986
       Ser Thr Arg Arg Ala Ala Gly Ala Ala Gln Pro Ala Arg Pro Thr Pro
                   270              275               280

       GCC TGC GCG CTT CCA TCA AGA AGG AGA CCA AGG TTC TCA AGA CCC TGT   1034
       Ala Cys Ala Leu Pro Ser Arg Arg Arg Pro Arg Phe Ser Arg Pro Cys
                   285              290               295

       CGG TGA TCA TGG GGG TCT TCG TGT GTT GCT GGC TGC CCT TCT TCA TCC   1082
 30    Arg  *  Ser Trp Gly Ser Ser Cys Val Ala Gly Cys Pro Ser Ser Ser
           300              305               310

       TTA ACT GCA TGG TCC CTT TCT GCA GTG GAC ACC CTG AAG GCC CTC CGG   1130
       Leu Thr Ala Trp Ser Leu Ser Ala Val Asp Thr Leu Lys Ala Leu Arg
           315              320               325               330

       CCG GCT TCC CCT GCG TCA GTG AGA CCA CCT TCG ACG TCT TCG TCT GGT   1178
 35    Pro Ala Ser Pro Ala Ser Val Arg Pro Pro Ser Thr Ser Ser Ser Gly
                   335              340               345
```

SUBSTITUTE SHEET

```
        TCG GCT GGG CTA ACT CCT CAC TCA ACC CCG TCA TCT ATG CCT TCA ACG   1226
        Ser Ala Gly Leu Thr Pro His Ser Thr Pro Ser Ser Met Pro Ser Thr
                        350             355             360
  5
        CCG ACT TTC AGA AGG TGT TTG CCC AGC TGC TGG GGT GCA GCC ACT TCT   1274
        Pro Thr Phe Arg Arg Cys Leu Pro Ser Cys Trp Gly Ala Ala Thr Ser
                        365             370             375

        GCT CCC GCA CGC CGG TGG AGA CGG TGA ACA TCA GCA ATG AGC TCA TCT   1322
        Ala Pro Ala Arg Arg Trp Arg Arg  *  Thr Ser Ala Met Ser Ser Ser
                        380             385             390
 10
        CCT ACA ACC AAG ACA TCG TCT TCC ACA AGG AAA TCG CAG CTG CCT ACA   1370
        Pro Thr Thr Lys Thr Ser Ser Ser Thr Arg Lys Ser Gln Leu Pro Thr
        395             400             405             410

        TCC ACA TGA TGC CCA ACG CCG TTA CCC CCG GCA ACC GGG AGG TGG ACA   1418
        Ser Thr  *  Cys Pro Thr Pro Leu Pro Pro Ala Thr Gly Arg Trp Thr
                        415             420             425
 15
        ACG ACG AGG AGG AGG GTC CTT TCG ATC GCA TGT TCC AGA TCT ATC AGA   1466
        Thr Thr Arg Arg Arg Val Leu Ser Ile Ala Cys Ser Arg Ser Ile Arg
                        430             435             440

        CGT CCC CAG ATG GTG ACC CTG TTG CTG AGT CTG TCT GGG AGC TGG ACT   1514
        Arg Pro Gln Met Val Thr Leu Leu Leu Ser Leu Ser Gly Ser Trp Thr
                        445             450             455
 20
        GCG AGG GGG AGA TTT CTT TAG ACA AAA TAA CAC CTT TCA CCC CGA ATG   1562
        Ala Arg Gly Arg Phe Leu  *  Thr Lys  *  His Leu Ser Pro Arg Met
                        460             465             470

        GAT TCC ATT AAA CTG CAT TAA GAA CCC TCA TGG ATC TGC ATA ACC GCA   1610
        Asp Ser Ile Lys Leu His  *  Glu Pro Ser Trp Ile Cys Ile Thr Ala
        475             480             485             490
 25
        CAG ACA CTG ACA AGC ACG CAC ACACACGCAA ATACATGCCT TTCAGTGCTG    1661
        Gln Thr Leu Thr Ser Thr His
                        495

        CTCCTTATCA TGTGTTCTGT GTAGTAGCTC GTGTGCTAGA ACTCACCATG ATGTCAGTCG  1721

        AGATGCAGAT CAGTGCATAC TCAGTCAAGT ATCAGCTACA GAGATGACAC             1771
 30
    (2) INFORMATION FOR SEQ ID NO:2:

            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 497 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear

 35     (ii) MOLECULE TYPE: protein
```

SUBSTITUTE SHEET

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
     Cys Cys Arg Gln Ala Ala Thr Ala Pro Arg Thr Arg Gly Ser Ser Leu
      1               5                  10                  15

     Tyr Thr Ser Ser Trp Arg Arg Gly Thr Pro Trp Gly Ala Arg Arg Gly
  5                   20                  25                  30

     His Arg His Trp Gly Pro His Arg Trp Ser Pro Pro Ala Cys  *  Pro
                  35                  40                  45

     Tyr Ser Ser Ser Gly Pro Cys Trp Ala Thr Cys Trp Cys Ala Gln Pro
          50                  55                  60

     Ser Cys Gly Ala Ala Thr Cys Ala Pro Thr  *  Pro Thr Ser Ser Ser
 10   65                  70                  75                  80

     Cys Leu Trp Pro Cys Gln Thr Phe Ser Trp Arg Cys Trp Ser Cys Pro
                  85                  90                  95

     Gly Arg Gln Ser Pro Arg Trp Pro Val Thr Gly Pro Leu Glu Arg Ser
                 100                 105                 110

     Ala Thr Ser Gly Trp Pro Ser Thr Ser Cys Ala Pro Leu Pro Pro Ser
 15              115                 120                 125

      *  Thr Cys Ala Ser Ser Ala Trp Thr Ala Thr Gly Pro Ser Pro Gly
         130                 135                 140

     Pro Ser Ala Thr Ser Ala Arg  *  Leu Ser Ala Trp Pro Trp Ser Trp
     145                 150                 155                 160
 20
     Ser Ala Trp His Gly Pro Cys Pro Ser Ser Ser Pro Ser Phe Arg Ser
                 165                 170                 175

     Ser Ser Thr Gly Thr Gly Thr Arg Arg Pro Leu Gly Ala Gly Trp Thr
                 180                 185                 190

     Cys Gln Thr Thr Trp Pro Thr Gly Arg Pro Gly Arg Arg Thr Phe Gly
                 195                 200                 205
 25
     Ser Pro Thr  *  Met Gln Arg Thr Val Thr Pro Ala  *  Ile Glu Pro
     210                 215                 220

     Thr Pro Ser Leu Pro Arg Ser Ser Ala Ser Thr Ser Pro Leu Pro Ser
     225                 230                 235                 240

      *  Ser  *  Pro Thr Arg Ala Ser Thr Ala Ser Pro Arg Cys Arg Ser
 30                  245                 250                 255

     Ala Gly Phe Pro Pro Trp Arg Gly Pro Gln Ser Thr Arg Arg Ala Ala
                 260                 265                 270

     Gly Ala Ala Gln Pro Ala Arg Pro Thr Pro Ala Cys Ala Leu Pro Ser

 35
```

				275					280				285		
5	Arg	Arg	Arg	Pro	Arg	Phe	Ser	Arg	Pro	Cys	Arg	*	Ser	Trp	Gly	Ser
		290					295				300					

Arg Arg Arg Pro Arg Phe Ser Arg Pro Cys Arg * Ser Trp Gly Ser
   290   295   300

Ser Cys Val Ala Gly Cys Pro Ser Ser Ser Leu Thr Ala Trp Ser Leu
305   310   315   320

Ser Ala Val Asp Thr Leu Lys Ala Leu Arg Pro Ala Ser Pro Ala Ser
        325   330   335

Val Arg Pro Pro Ser Thr Ser Ser Gly Ser Ala Gly Leu Thr Pro
        340   345   350

His Ser Thr Pro Ser Ser Met Pro Ser Thr Pro Thr Phe Arg Arg Cys
        355   360   365

Leu Pro Ser Cys Trp Gly Ala Ala Thr Ser Ala Pro Ala Arg Arg Trp
    370   375   380

Arg Arg * Thr Ser Ala Met Ser Ser Ser Pro Thr Thr Lys Thr Ser
385   390   395   400

Ser Ser Thr Arg Lys Ser Gln Leu Pro Thr Ser Thr * Cys Pro Thr
            405   410   415

Pro Leu Pro Pro Ala Thr Gly Arg Trp Thr Thr Thr Arg Arg Arg Val
        420   425   430

Leu Ser Ile Ala Cys Ser Arg Ser Ile Arg Arg Pro Gln Met Val Thr
        435   440   445

Leu Leu Leu Ser Leu Ser Gly Ser Trp Thr Ala Arg Gly Arg Phe Leu
    450   455   460

* Thr Lys * His Leu Ser Pro Arg Met Asp Ser Ile Lys Leu His
465   470   475   480

* Glu Pro Ser Trp Ile Cys Ile Thr Ala Gln Thr Leu Thr Ser Thr
        485   490   495

His

(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 480 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
   Met Leu Pro Pro Gly Ser Asn Gly Thr Ala Tyr Pro Gly Gln Phe Ala
5  1               5                   10                  15

   Leu Tyr Gln Gln Leu Ala Gln Gly Asn Ala Val Gly Gly Ser Ala Gly
            20                  25                  30

   Ala Pro Pro Leu Gly Pro Ser Gln Val Val Thr Ala Cys Leu Leu Thr
            35              40                  45

10 Leu Leu Ile Ile Trp Thr Leu Leu Gly Asn Val Leu Val Cys Ala Ala
       50              55                  60

   Ile Val Arg Ser Arg His Leu Arg Ala Asn Met Thr Asn Val Phe Ile
   65              70                  75                  80

   Val Ser Leu Ala Val Ser Asp Leu Phe Val Ala Leu Leu Val Met Pro
                85                  90                  95

15 Trp Lys Ala Val Ala Glu Val Ala Gly Tyr Trp Ala Phe Gly Ala Phe
            100                 105                 110

   Gly Ala Phe Cys Asp Val Trp Val Ala Phe Asp Ile Met Cys Ser Thr
            115                 120                 125

   Ala Ser Ile Leu Asn Leu Cys Val Ile Ser Val Asp Arg Tyr Trp Ala
       130                 135                 140
20
   Ile Ser Arg Pro Phe Arg Tyr Lys Arg Lys Met Thr Gln Arg Met Ala
   145                 150                 155                 160

   Leu Val Met Val Gly Leu Ala Trp Thr Leu Ser Ile Leu Ile Ser Phe
                165                 170                 175

   Ile Pro Val Gln Leu Asn Trp His Arg Asp Gln Ala Ala Ser Trp Gly
25                  180                 185                 190

   Gly Leu Asp Leu Pro Asn Asn Leu Ser Asn Glu Tyr Pro Glu Arg Arg
            195                 200                 205

   Phe Gly Glu Arg Pro Phe Asx Asn Ser Arg Asn Val Phe Asp Asp Leu
            210                 215                 220

30 Asn Thr Tyr Tyr Ala Ile Asp Asp Asp Leu Ile Asp Gly Tyr Ile Pro
   225                 230                 235                 240

   Asx Ser Ile Met Ile Asx Tyr Tyr Tyr Thr Ile Tyr Thr Ile Ser Trp
                245                 250                 255

   Asx Trp Ile Thr Thr Ile Asp Asp Leu Arg Thr Ser Ser Arg His Ser
                260                 265                 270
35
```

```
       Trp Asp Val Thr Asp Asp Ser Ser Val Ser Pro Phe Tyr Asp Leu Thr
               275             280             285

       Ser Asp Ile Lys Lys Arg Tyr Lys Asx Leu Lys Tyr Leu Asp Asx Ile
               290             295             300

  5    Met His Asx Gly Asx Val Val Glu Leu Pro Gly Gly Ile Leu Asn Val
               305             310             315             320

       Met Asx Pro Gly Val Asp His His Pro Arg His Pro Pro Ser His Gly
                       325             330             335

       Pro Val Asx Asp Arg Tyr Tyr Gly Phe Asx Gly Asx Glu Gly His Glu
                       340             345             350

  10   Ser Asn Asp Asp Leu Asn Pro Asx Ile Tyr Ser Gly Asn Ser Phe Gly
                       355             360             365

       Trp Lys Asx Gly Ser Trp Leu Leu His Val Asp His Gly Val Asp Thr
               370             375             380

       Tyr Pro Asx Arg Tyr Asx Asn Ile Asp Asn Arg Leu Ile Asp Tyr Asn
           385             390             395             400

  15   Trp Phe Ile Asx Gly His Lys Arg Ile Ser Ser Ser Tyr Ile His Met
                       405             410             415

       Met Pro Asn Ser Asx Tyr Pro His Asn Thr Arg Asx Phe Asn Phe Arg
                       420             425             430

       Arg Arg His Pro Gly Phe Thr Met Gly Trp Ile Tyr Trp Tyr Asp Pro
               435             440             445

  20

       Phe His Phe Pro Asx Ser Arg Asp Asx Glu Arg Leu Phe Val Arg His
           450             455             460

       Arg Ile Asp Leu Phe Lys Ile Tyr Pro Gly Tyr Pro Asn His Gly His
           465             470             475             480
```

  35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
     Met Leu Pro Pro Arg Ser Asn Gly Thr Ala Tyr Pro Gly Gln Leu Ala
     1               5                   10                  15

  5  Leu Tyr Gln Gln Leu Ala Gln Gly Asn Ala Val Gly Gly Ser Ala Gly
                 20                  25                  30

     Ala Pro Pro Leu Gly Pro Val Gln Val Val Thr Ala Cys Leu Leu Thr
             35                  40                  45

     Leu Leu Ile Ile Trp Thr Leu Leu Gly Asn Val Leu Met Ser Ala Ala
         50                  55                  60
 10
     Ile Val Arg Thr Arg His Leu Arg Ala Lys Met Thr Asn Val Phe Ile
     65                  70                  75                  80

     Val Ser Leu Ala Val Ser Asp Leu Phe Val Ala Leu Leu Val Met Pro
                 85                  90                  95

     Trp Lys Ala Val Ala Glu Val Ala Gly Tyr Trp Pro Phe Glu Ala Phe
 15              100                 105                 110

     Cys Asp Val Trp Val Ala Phe Asp Ile Met Cys Ser Thr Ala Ser Ile
             115                 120                 125

     Leu Asn Leu Cys Val Ser Val Ile Ser Val Gly Arg Tyr Trp Ala Ile
         130                 135                 140

 20  Ser Arg Pro Phe Arg Tyr Glu Arg Lys Met Thr Gln Arg Met Ala Leu
     145                 150                 155                 160

     Val Met Val Gly Pro Ala Trp Thr Leu Ser Ser Leu Ile Ser Phe Ile
                 165                 170                 175

     Pro Val Gln Leu Asn Trp His Arg Asp Gln Ala Val Ser Gly Gly Leu
                 180                 185                 190
 25
     Asp Leu Pro Asn Asn Leu Ser Asn Glu Tyr Pro Glu Arg Arg Ser Asx
             195                 200                 205

     Glu Arg Pro Phe Asx Thr Ser Arg Asn Val Phe Asp Asp Leu Asn Thr
         210                 215                 220

     Tyr Tyr Ser Ile Asp Asp Asp Leu Ile Asn Gly Tyr Ile Pro Met Ser
 30  225                 230                 235                 240

     Ile Met Ile Asx Tyr Tyr Tyr Thr Ile Tyr Thr Ile Ser Trp Asx Trp
                 245                 250                 255

     Ile Val Thr Ile Asp Asp Leu Arg Thr Ser Ser Arg His Asx Trp Asp
                 260                 265                 270

 35  Val Thr Asp Asp Ser His Val Tyr Pro Thr Ser Leu Arg Phe Ser Ile
```

                    275   280   285
       Lys Lys Arg Tyr Lys Asx Leu Lys Pro Leu Asp Asx Ile Met His Asx
           290   295   300
  5
       Gly Asx Val Val Glu Leu Pro Gly Gly Ile Leu Asn Val Met Asx Pro
       305   310   315   320
       Gly Thr Asp His His Pro Lys His Pro Pro Ser Gly His Pro Lys Gly
                       325   330   335
       Pro Pro Ala Gly Phe Pro Cys Val Ser Glu Thr Thr Phe Asp Val Phe
               340   345   350
 10
       Ile Trp Phe Cys Trp Ala Asn Ser Ser Leu Asn Pro Val Tyr Ala Phe
           355   360   365
       Asn Ala Asp Phe Trp Lys Val Phe Ala Gln Leu Leu Gly Cys Ser His
       370   375   380
 15
       Val Cys Ser Arg Thr Pro Val Glu Thr Val Asn Ile Ser Asn Glu Leu
       385   390   395   400
       Ile Ser Tyr Asn Gln Asp Met Val Phe His Lys Glu Ile Ala Ala Ala
                   405   410   415
       Cys Ile His Met Met Pro Asn Ala Val Pro Pro Gly Asp Gln Glu Val
                   420   425   430
 20
       Asp Asn Asp Glu Glu Glu Glu Ser Pro Phe Asp Arg Met Ser Gln Ile
               435   440   445
       Tyr Gln Thr Ser Pro Asp Gly Asp Pro Val Ala Glu Ser Val Glu Leu
           450   455   460
       Asp Cys Glu Gly Glu Ile Ser Leu Asp Lys Ile Thr Pro Phe Thr Pro
       465   470   475   480
 25
       Asn Gly Phe His

  (2) INFORMATION FOR SEQ ID NO:5:

       (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 446 amino acids
 30   (B) TYPE: amino acid
           (C) STRANDEDNESS: single
           (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: peptide

     (vii) IMMEDIATE SOURCE:
 35   (B) CLONE: D1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Arg Thr Leu Asn Thr Ser Ala Met Asp Gly Thr Gly Leu Val Val
1               5               10              15

Glu Arg Asp Phe Ser Val Arg Ile Leu Thr Ala Cys Phe Leu Ser Leu
            20              25              30

Leu Ile Leu Ser Thr Leu Leu Gly Asn Thr Leu Val Cys Ala Ala Val
        35              40              45

Ile Arg Phe Arg His Leu Arg Ser Lys Val Thr Asn Phe Phe Val Ile
    50              55              60

Ser Leu Ala Val Ser Asp Leu Leu Val Ala Val Leu Val Met Pro Trp
65              70              75              80

Lys Ala Val Ala Glu Ile Ala Gly Phe Trp Pro Phe Gly Ser Phe Cys
            85              90              95

Asn Ile Trp Val Ala Phe Asp Ile Met Cys Ser Thr Ala Ser Ile Leu
        100             105             110

Asp Leu Cys Val Ile Ser Val Asp Arg Tyr Trp Ala Ile Ser Ser Pro
        115             120             125

Phe Arg Tyr Glu Arg Lys Met Thr Pro Lys Ala Ala Phe Ile Leu Ile
    130             135             140

Ser Val Ala Trp Thr Leu Ser Val Leu Ile Ser Phe Ile Pro Val Gln
145             150             155             160

Leu Ser Trp His Lys Ala Lys Pro Thr Ser Pro Ser Asp Gly Asn Ala
            165             170             175

Thr Ser Leu Ala Glu Thr Ile Asp Asn Cys Asp Ser Ser Leu Ser Arg
            180             185             190

Thr Tyr Ala Ile Ser Ser Ser Val Ile Ser Phe Tyr Ile Pro Val Ala
        195             200             205

Ile Met Ile Val Thr Tyr Thr Arg Ile Tyr Arg Ile Ala Gln Lys Gln
    210             215             220

Ile Arg Arg Ile Ala Ala Leu Glu Arg Ala Ala Val His Ala Lys Asn
225             230             235             240

Cys Gln Thr Thr Thr Gly Asn Gly Lys Pro Val Glu Cys Ser Gln Pro
            245             250             255

Glu Ser Ser Phe Lys Met Ser Phe Lys Arg Glu Thr Lys Val Leu Lys
            260             265             270
```

-63-

```
 5


     Thr Leu Ser Val Ile Met Gly Val Phe Val Cys Cys Trp Leu Pro Phe
             275                 280                 285
10
     Phe Ile Leu Asn Cys Ile Leu Pro Phe Cys Gly Ser Gly Glu Thr Gln
             290                 295                 300

     Pro Phe Cys Ile Asp Ser Asn Thr Phe Asp Val Phe Val Trp Phe Gly
     305                 310                 315                 320

     Trp Ala Asn Ser Ser Leu Asn Pro Ile Ile Tyr Ala Phe Asn Ala Asp
15                   325                 330                 335

     Phe Arg Lys Ala Phe Ser Thr Leu Leu Gly Cys Tyr Arg Leu Cys Pro
                 340                 345                 350

     Ala Thr Asn Asn Ala Ile Glu Thr Val Ser Ile Asn Asn Asn Gly Ala
             355                 360                 365

20   Ala Met Phe Ser Ser His His Glu Pro Arg Gly Ser Ile Ser Lys Glu
             370                 375                 380

     Cys Asn Leu Val Tyr Leu Ile Pro His Ala Val Gly Ser Ser Glu Asp
     385                 390                 395                 400

     Leu Lys Lys Glu Glu Ala Ala Gly Ile Ala Arg Pro Leu Glu Lys Leu
                 405                 410                 415
25
     Ser Pro Ala Leu Ser Val Ile Leu Asp Tyr Asp Thr Asp Val Ser Leu
                 420                 425                 430

     Glu Lys Ile Gln Pro Ile Thr Gln Asn Gly Gln His Pro Thr
                 435                 440                 445


30



35
```

What is claimed is:

1. An isolated nucleic acid molecule encoding a human dopamine D_1 receptor.

2. An isolated DNA molecule of claim 1.

3. A cDNA molecule of claim 2 encoding a human dopamine D_1 receptor having a coding sequence substantially the same as the coding sequence shown in Figure 1.

4. An isolated protein which is a human dopamine D_1 receptor.

5. An isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1.

6. A vector comprising the DNA molecule of claim 2.

7. A plasmid of claim 6.

8. A plasmid of claim 7 designated pdopD1-GL-30 (ATCC Accession No. 40839).

9. A vector of claim 6 adapted for expression in a bacterial cell which comprises the regulatory elements necessary for expression of the DNA in the bacterial cell so located relative to the DNA encoding the dopamine D_1 receptor as to permit expression thereof.

10. A vector of claim 6 adapted for expression in a yeast

cell which comprises the regulatory elements necessary
for expression of the DNA in the yeast cell so located
relative to the DNA encoding the dopamine D_1 receptor
as to permit expression thereof.

5

11. A vector of claim 6 adapted for expression in a
mammalian cell which comprises the regulatory elements
necessary for expression of the DNA in the mammalian
cell so located relative to the DNA encoding the
10 dopamine D_1 receptor as to permit expression thereof.

12. A plasmid of claim 7 adapted for expression in a
mammalian cell which comprises the regulatory elements
necessary for expression of the DNA in the mammalian
15 cell so located relative to the DNA encoding the
dopamine D_1 receptor as to permit expression thereof.

13. A plasmid comprising the cDNA molecule of claim 3
adapted for expression in a mammalian cell which
20 comprises the regulatory elements necessary for
expression of the DNA in the mammalian cell so located
relative to the cDNA molecule as to permit expression
thereof.

25   14. A mammalian cell comprising the plasmid of claim 7.

15. An Ltk- cell comprising the plasmid of claim 7.

16. An Ltk- cell comprising the plasmid of claim 13.

30

17. A method for determining whether a ligand not known to
be capable of binding to a human dopamine D_1 receptor
can bind to a human dopamine D_1 receptor which

comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a human dopamine D_1 receptor with the ligand under conditions permitting binding of ligands known to bind to a dopamine D_1 receptor, detecting the presence of any of the ligand bound to a human dopamine D_1 receptor, and thereby determining whether the ligand binds to a human dopamine D_1 receptor.

18. The method of claim 17 wherein the mammalian cell is nonneuronal in origin.

19. A method of claim 18, wherein the mammalian cell nonneuronal in origin is an Ltk- cell.

20. A ligand detected by the method of claim 17.

21. A method of screening drugs to identify drugs which specifically interact with, and bind to, the human dopamine D_1 receptor on the surface of a cell which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a human dopamine D_1 receptor with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, a human dopamine D_1 receptor.

22. The method of claim 21 wherein the mammalian cell is nonneuronal in origin.

23. The method of claim 22 wherein the mammalian cell nonneuronal in origin is an Ltk- cell.

24. A pharmaceutical composition comprising a drug identified by the method of claim 21 and a pharmaceutically acceptable carrier.

25. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human dopamine D_1 receptor.

26. A DNA probe comprising a DNA molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence shown in Figure 1.

27. A method of detecting expression of a dopamine D_1 receptor on the surface of a cell by detecting the presence of mRNA coding for a dopamine D_1 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the nucleic acid probe of claim 25 under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the dopamine D_1 receptor by the cell.

28. An antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a human dopamine D_1 receptor so as to prevent translation of the mRNA molecule.

29. An antisense oligonucleotide having a sequence capable of binding specifically with any sequences of the cDNA molecule of claim 3.

30.   An antisense oligonucleotide of claim 28 comprising chemical analogues of nucleotides.

31.   A pharmaceutical composition comprising an amount of the oligonucleotide of claim 28 effective to reduce expression of a human dopamine D_1 receptor by passing through a cell membrane and binding specifically with mRNA encoding a human dopamine D_1 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane.

32.   A pharmaceutical composition of claim 31, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

33.   A pharmaceutical composition of claim 32, wherein the substance which inactivates mRNA is a ribozyme.

34.   A pharmaceutical composition of claim 31, wherein the pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane comprises a structure which binds to a receptor specific for a selected cell type and is thereby taken up by cells of the selected cell type.

35.   A method of treating abnormalities which are alleviated by reduction of expression of a dopamine D_1 receptor which comprises administering to a subject an amount of the pharmaceutical composition of claim 31 effective to reduce expression of the dopamine D_1 receptor by the subject.

-69-

36.   A method of treating an abnormal condition related to an excess of dopamine D_1 receptor activity which comprises administering to a subject an amount of the pharmaceutical composition of claim 31 effective to reduce expression of the dopamine D_1 receptor by the subject.

5

37.   The method of claim 36 wherein the abnormal condition is dementia.

10

38.   The method of claim 36 wherein the abnormal condition Parkinson's disease.

39.   The method of claim 36 wherein the abnormal condition is abnormal cognitive functioning.

15

40.   The method of claim 36 wherein the abnormal condition is schizophrenia.

20

41.   The method of claim 36 wherein the abnormal condition is tardive dyskinesia.

42.   The method of claim 36 wherein the abnormal condition is renal failure.

25

43.   The method of claim 36 wherein the abnormal condition is failure of vascular control.

44.   The method of claim 36 wherein the abnormal condition is abnormal circadian rhythms.

30

45.   The method of claim 36 wherein the abnormal condition is abnormal visual activity.

46. An antibody directed to a human dopamine D_1 receptor.

47. A monoclonal antibody directed to an epitope of a human dopamine D_1 receptor present on the surface of a cell and having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human dopamine D_1 receptor included in the amino acid sequence shown in Figure 1.

48. A pharmaceutical composition which comprises an amount of the antibody of claim 46 effective to block binding of naturally occurring ligands to the dopamine D_1 receptor and a pharmaceutically acceptable carrier.

49. A method of treating abnormalities which are alleviated by reduction of expression of a human dopamine D_1 receptor which comprises administering to a subject an amount of the pharmaceutical composition of claim 48 effective to block binding of naturally occurring ligands to the dopamine D_1 receptor and thereby alleviate abnormalities resulting from overexpression of a human dopamine D_1 receptor.

50. A method of treating an abnormal condition related to an excess of dopamine D_1 receptor activity which comprises administering to a subject an amount of the pharmaceutical composition of claim 48 effective to block binding of naturally occurring ligands to the dopamine D_1 receptor and thereby alleviate the abnormal condition.

51. The method of claim 50 wherein the abnormal condition is dementia.

52. The method of claim 50 wherein the abnormal condition Parkinson's disease.

53. The method of claim 50 wherein the abnormal condition is abnormal cognitive functioning.

54. The method of claim 50 wherein the abnormal condition is schizophrenia.

55. The method of claim 50 wherein the abnormal condition is tardive dyskinesia.

56. The method of claim 50 wherein the abnormal condition is renal failure.

57. The method of claim 50 wherein the abnormal condition is failure of vascular control.

58. The method of claim 50 wherein the abnormal condition is abnormal circadian rhythms.

59. The method of claim 50 wherein the abnormal condition is abnormal visual activity.

60. A method of detecting the presence of a human dopamine D_1 receptor on the surface of a cell which comprises contacting the cell with the antibody of claim 46 under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a human dopamine D_1 receptor on the surface of the cell.

61. A transgenic nonhuman mammal expressing DNA encoding a

human dopamine D_1 receptor.

62. A transgenic nonhuman mammal expressing DNA encoding a human dopamine D_1 receptor so mutated as to be incapable of normal receptor activity, and not expressing native dopamine D_1 receptor.

63. A transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a human dopamine D_1 receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a dopamine D_1 receptor and which hybridizes to mRNA encoding a dopamine D_1 receptor thereby reducing its translation.

64. The transgenic nonhuman mammal of any of claims 61, 62, or 63, wherein the DNA encoding a human dopamine D_1 receptor additionally comprises an inducible promoter.

65. The transgenic nonhuman mammal of any of claims 61, 62, or 63, wherein the DNA encoding a human dopamine D_1 receptor additionally comprises tissue specific regulatory elements.

66. A transgenic mouse of any of claims 61, 62, or 63.

67. A method of determining the physiological effects of expressing varying levels of human dopamine D_1 receptors which comprises producing a transgenic nonhuman animal whose levels of human dopamine D_1 receptor expression are varied by use of an inducible promoter which regulates human dopamine D_1 receptor expression.

68. A method of determining the physiological effects of expressing varying levels of human dopamine D_1 receptors which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human dopamine D_1 receptor.

69. A method for identifying a substance capable of alleviating the abnormalities resulting from overexpression of a human dopamine D_1 receptor comprising administering a substance to the transgenic nonhuman mammal of claim 61 and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overexpression of a human dopamine D_1 receptor.

70. A pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a human dopamine D_1 receptor and a pharmaceutically acceptable carrier.

71. A method for treating the abnormalities resulting from overexpression of a human dopamine D_1 receptor which comprises administering to a subject an amount of the pharmaceutical composition of claim 70 effective to alleviate the abnormalities resulting from overexpression of a human dopamine D_1 receptor.

72. A method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a human dopamine D_1 receptor comprising administering the substance to the transgenic nonhuman mammal of either of claims 62 or 63

and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underexpression of a human dopamine D_1 receptor.

73. A pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of dopamine D_1 receptor and a pharmaceutically acceptable carrier.

74. A method for treating the abnormalities resulting from underexpression of a human dopamine D_1 receptor which comprises administering to a subject an amount of the pharmaceutical composition of claim 73 effective to alleviate the abnormalities resulting from underexpression of a human dopamine D_1 receptor.

75. A method for diagnosing in a subject a predisposition to a disorder associated with the expression of a specific human dopamine D_1 receptor allele which comprises:

a. isolating DNA from victims of the disorder;

b. digesting the isolated DNA of step a with at least one restriction enzyme;

c. electrophoretically separating the resulting DNA fragments on a sizing gel;

d. contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human dopamine D_1 receptor and labelled

with a detectable marker;

  e. detecting labelled bands on the gel which have
5   hybridized to the DNA encoding a human dopamine D_1
      receptor labelled with a detectable marker to
      create a band pattern specific to the DNA of
      victims of the disorder;

  f. preparing the subject's DNA by steps a-e to
10   produce detectable labelled bands on a gel; and

  g. comparing the band pattern specific to the DNA of
      victims of the disorder of step e and the
      subject's DNA of step f to determine whether the
15   patterns are the same or different and to
      diagnose thereby predisposition to the disorder
      if the patterns are the same.

76. The method of claim 75 wherein a disorder associated
20 with the expression of a specific human dopamine D_1
    receptor allele is diagnosed.

77. A method of preparing the isolated dopamine D_1 receptor
    of claim 4 which comprises:
25

  a. inducing cells to express dopamine D_1 receptor;

  b. recovering the receptor from the resulting cells;
      and
30

  c. purifying the receptor so recovered.

78. A method of preparing the isolated dopamine D_1 receptor

of claim 4 which comprises:

a.   inserting nucleic acid encoding dopamine D_1 receptor in a suitable vector;

5

b.   inserting the resulting vector in a suitable host cell;

c.   recovering the receptor produced by the resulting

10   cell; and

d.   purifying the receptor so recovered.

```
         -130                    -110                    -90
          .        .        .        .        .        .        .
GCAGCTCATGGTGACCCCCCTCTGGGCTCGAGGGTCCCTTGGCTGAGGGGGCGCATCCTC
          -70                    -50                    -30
          .        .        .        .        .        .        .
GGGGTGCCGATGGGGCTGCCTGGGGGTCGCAGGGCTGAAGTTGGGACCGCGCACAGACCG
          -10                     10                     30
          .        .        .        .        .        .        .
CCCCTGCAGTCCAGCCCAAATGCTGCCGCCAGGCAGCAACGGCACCGCGTACCCGGGGCA
                          M   L   P   P   G   S   N   G   T   A   Y   P   G   Q
            50                      70                     90
          .        .        .        .        .        .        .
GTTCGCTCTATACCAGCAGCTGGCGCAGGGGAACGCCGTGGGGGGCTCGGCGGGGGCACC
  F   A   L   Y   Q   Q   L   A   Q   G   N   A   V   G   G   S   A   G   A   P
            110                     130                    150
          .        .        .        .        .        .        .
GCCACTGGGGCCCTCACAGGTGGTCACCGCCTGCCTGCTGACCCTACTCATCATCTGGAC
  P   L   G   P   S   Q   V   V   T   A   C   L   L   T   L   L   I   I   W   T
            170                     190                    210
          .        .        .        .        .        .        .
CCTGCTGGGCAACGTGCTGGTGTGCGCAGCCATCGTGCGGAGCCGCCACCTGCGCGCCAA
  L   L   G   N   V   L   V   C   A   A   I   V   R   S   R   H   L   R   A   N
            230                     250                    270
          .        .        .        .        .        .        .
CATGACCAACGTCTTCATCGTGTCTCTGGCCGTGTCAGACCTTTTCGTGGCGCTGCTGGT
  M   T   N   V   F   I   V   S   L   A   V   S   D   L   F   V   A   L   L   V
```

FIGURE 1A

SUBSTITUTE SHEET

2/10

290   310   330

CATGCCCTGGAAGGCAGTCGCCGAGGTGGCCGGTTACTGGGCCTTTGGAGCGTTCTGCGA

M   P   W   K   A   V   A   E   V   A   G   Y   W   A   F   G   A   F   C   D

350   370   390

CGTCTGGGTGGCCTTCGACATCATGTGCTCCACTGCCTCCATCCTGAACCTGTGCGTCAT

V   W   V   A   F   D   I   M   C   S   T   A   S   I   L   N   L   C   V   I

410   430   450

CAGCGTGGACCGCTACTGGGCCATCTCCAGGCCCTTCCGCTACAAGCGCAAGATGACTCA

S   V   D   R   Y   W   A   I   S   R   P   F   R   Y   K   R   K   M   T   Q

470   490   510

GCGCATGGCCTTGGTCATGGTCGGCCTGGCATGGACCTTGTCCATCCTCATCTCCTTCAT

R   M   A   L   V   M   V   G   L   A   W   T   L   S   I   L   I   S   F   I

530   550   570

TCCGGTCCAGCTCAACTGGCACAGGGACCAGGCGGCCTCTTGGGGCGGGCTGGACCTGCC

P   V   Q   L   N   W   H   R   D   Q   A   A   S   W   G   G   L   D   L   P

590   610   630

AAACAACCTGGCCAACTGGACGCCCTGGGAGGAGGACTTTTGGGAGCCCGACGTGAATGC

N   N   L   A   N   W   T   P   W   E   E   D   F   W   E   P   D   V   N   A

650   670   690

AGAGAACTGTGACTCCAGCCTGAATCGAACCTACGCCATCTCTTCCTCGCTCATCAGCTT

FIGURE 1B

SUBSTITUTE SHEET

E   N   C   D   S   S   L   N   R   T   Y   A   I   S   S   S   L   I   S   F
    710   730   750

CTACATCCCCGTTGCCATCATGATCGTGACCTACACGCGCATCTACCGCATCGCCCAGGT
Y   I   P   V   A   I   M   I   V   T   Y   T   R   I   Y   R   I   A   Q   V
    770   790   810

GCAGATCCGCAGGATTTCCTCCCTGGAGAGGGCCGCAGAGCACGCGCAGAGCTGCCGGAG
Q   I   R   R   I   S   S   L   E   R   A   A   E   H   A   Q   S   C   R   S
    830   850   870

CAGCGCAGCCTGCGCGCCCGACACCAGCCTGCGCGCTTCCATCAAGAAGGAGACCAAGGT
S   A   A   C   A   P   D   T   S   L   R   A   S   I   K   K   E   T   K   V
    890   910   930

TCTCAAGACCCTGTCGGTGATCATGGGGGGTCTTCGTGTGTTGCTGGCTGCCCTTCTTCAT
L   K   T   L   S   V   I   M   G   V   F   V   C   C   W   L   P   F   F   I
    950   970   990

CCTTAACTGCATGGTCCCTTTCTGCAGTGGACACCCTGAAGGCCCTCCGGCCGGCTTCCC
L   N   C   M   V   P   F   C   S   G   H   P   E   G   P   P   A   G   F   P
    1010   1030   1050

CTGCGTCAGTGAGACCACCTTCGACGTCTTCGTCTGGTTCGGCTGGGCTAACTCCTCACT
C   V   S   E   T   T   F   D   V   F   V   W   F   G   W   A   N   S   S   L
    1070   1090   1110

FIGURE 1C

SUBSTITUTE SHEET

CAACCCCGTCATCTATGCCTTCAACGCCGACTTTCAGAAGGTGTTTGCCCAGCTGCTGGG

N   P   V   I   Y   A   F   N   A   D   F   Q   K   V   F   A   Q   L   L   G

    1130   1150   1170

GTGCAGCCACTTCTGCTCCCGCACGCCGGTGGAGACGGTGAACATCAGCAATGAGCTCAT

C   S   H   F   C   S   R   T   P   V   E   T   V   N   I   S   N   E   L   I

    1190   1210   1230

CTCCTACAACCAAGACATCGTCTTCCACAAGGAAATCGCAGCTGCCTACATCCACATGAT

S   Y   N   Q   D   I   V   F   H   K   E   I   A   A   A   Y   I   H   M   M

    1250   1270   1290

GCCCAACGCCGTTACCCCCGGCAACCGGGAGGTGGACAACGACGAGGAGGAGGGTCCTTT

P   N   A   V   T   P   G   N   R   E   V   D   N   D   E   E   E   G   P   F

    1310   1330   1350

CGATCGCATGTTCCAGATCTATCAGACGTCCCCAGATGGTGACCCTGTTGCTGAGTCTGT

D   R   M   F   Q   I   Y   Q   T   S   P   D   G   D   P   V   A   E   S   V

    1370   1390   1410

CTGGGAGCTGGACTGCGAGGGGGAGATTTCTTTAGACAAAATAACACCTTTCACCCCGAA

W   E   L   D   C   E   G   E   I   S   L   D   K   I   T   P   F   T   P   N

    1430   1450   1470

TGGATTCCATTAAACTGCATTAAGAACCCTCATGGATCTGCATAACCGCACAGACACTGA

G   F   H   *

    1490   1510   1530

FIGURE 1D

SUBSTITUTE SHEET

CAAGCACGCACACACACGCAAATACATGCCTTTCAGTGCTGCTCCTTATCATGTGTTCTG

1550   1570   1590

TGTAGTAGCTCGTGTGCTAGAACTCACCATGATGTCAGTCGAGATGCAGATCAGTGCATA

1610   1630

CTCAGTCAAGTATCAGCTACAGAGATGACAC

FIGURE 1E

SUBSTITUTE SHEET

6/10 Figure 2	7/10 Figure 2 ---cont'd---	8/10 Figure 2 ---cont'd---	9/10 Figure 2 ---cont'd---	10/10 Figure 2 ---cont'd---

6/10

FIGURE 2

```
GL-30   MLPPGSNGTAYPGQFALYQQLAQGN   25
GL-39   MLPPRSNGTAYPGQLALYQQLAQGN   25
D1                    MRTLNTSA    8

GL-30   AVGGSAGAPPLGPSQVVTACLLTLL   50
GL-39   AVGGSAGAPPLGPVQVVTACLLTLL   50
D1      MDGTGLVVERDFSVRILTACFLSLL   33
                                    I

GL-30   IIWTLLGNVLVCAA·IVRSRHLRAN   74
GL-39   IIWTLLGNVLMSAA·IVRTRHLRAK   74
D1      ILSTLLGNTLVCAAV·I·RFRHLRSK  58
                                    II

GL-30   MTNVFIVSLAVSDLFVALLVMPWKA   99
GL-39   MTNVFIVSLAVSDLFVALLVMPWKA   99
D1      VTNFFVISLAVSDLLVAVLVMPWKA   82

GL-30   VAEVAGYWAFGAFCDVWVAFDIMCS  124
GL-39   VAEVAGYWPFEAFCDVWVAFDIMCS  124
D1      VAEIAGFWPFGSFCNIWVAFDIMCS  107
```

III →

IV

		147 / 149 / 130
GL-30	T A S I L N L C V . I S V D R Y W A I S R P F R	147
GL-39	T A S I L N L C V S V I S V G R Y W A I S R P F R	149
D1	T A S I L D L C V . I S V D R Y W A I S P F R	130

GL-30	Y K R K M T Q R M A L V M V G L A W T L S I L I S	172
GL-39	Y E R K M T Q R M A L V M V G P A W T L S L I S	174
D1	Y E R K M T P K A A F I L I S V A W T L S V L I S	155

GL-30	F I P V Q L N W H R D Q A A S W G G L D L P N N L	197
GL-39	F I P V Q L N W H R D Q A V S * G G L D L P N N L	199
D1	F I P V Q L S W H K A K P T S . P S D G . . .	174

GL-30	A N W T P W E E D F W E P D V N A E N C D S S L N	222
GL-39	A N W T P W E E A V W E P D V R A E N C D S S L N	224
D1	. N A T S L A E . . . . T I D N C D S S L S	191

SUBSTITUTE SHEET

Protein sequence alignment (regions V and VI) for GL-30, GL-39, and D1.

```
                                                                        247
            V
GL-30  RTYAISSLISFYIPVAIMIVTYTR                                         247
GL-39  RTYAISSLINFYIPMAIMIVTYTR                                         249
D1     RTYAISSVISFYIPVAIMIVTYTR                                         216

GL-30  IYRIAQV.QIRRISSLERAAAEHAQS                                       271
GL-39  IYRIAQV.QICRISSLERAAEHVQS                                        273
D1     IYRIAQK.QIRRIAALERAAVHAKN                                        240

GL-30  CRSS.....ACAPDTSLRASIK                                           289
GL-39  CRSS.....AGCTPDTSLRFSIK                                          291
D1     CQTTTGNGKPVECSQPESSFKMSFK                                        265
                        VI
GL-30  KETKVLKTLSVIMGVFVCCWLPFFI                                        314
GL-39  KETKVLKPLSVIMGVFVCCWLPFFI                                        316
D1     RETKVLKTLSVIMGVFVCCWLPFFI                                        290

GL-30  LNCMVPFC.SGHPEGPPAGFPCV.S                                        337
GL-39  LNCMVPFF.RSGHPKGPPAGFPCV.S                                       339
D1     LNCILPFCGSG..ETQP...F.CIDS                                       310
```

VII

GL-30 E T T F D V F V W F G W A N S S L N P V I Y A F N 362
GL-39 E T T F D V F I W F C W A N S S L N P V Y A F N 363
D1   N T . F D V F V W F G W A N S S L N P I Y A F N 334

GL-30 A D F Q K V F A Q L L G C S H F C S R T . . P V E 385
GL-39 A D F W K V F A Q L L G C S H V C S R T . . P V E 386
D1   A D F R K A F S T L L G C Y R L C P A T N N A I E 359

GL-30 T V N I S N E L I S Y N Q D I V F H K E I A A A Y 410
GL-39 T V N I S N E L I S Y N Q D M V F H K E I A A A C 411
D1   T V S I N N G . A A M F S S . H H . E P R G . S 380

GL-30 I H M M P N A V T P G N R E V D N D E . . . E E 431
GL-39 I H M M P N A V P P G D Q E V D N D E . . . E E 433
D1   I S K E C N L V Y L I P H A V G S S E D L K K E 406

SUBSTITUTE SHEET

```
GL-30   G P F D . R M F Q I Y Q T S P D G D P V A E S V W   455
GL-39   S P F D . R M S Q I Y Q T S P D G D P V A E S V *   457
D1      A A G I A R P L E K L . S P . . . . A L S V I       424

GL-30   E L D C E G E I S L D K I T P F T P N G F H *       477
GL-39   E L D C E G E I S L D K I T P F T P N G F H *       480
D1      . L D Y D T D V S L E K I Q P I T Q N G Q H P T *   447
```

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C07H 15/12; C12N 15/00; C12P 21/06
U.S. Cl: 536/27; 435/172.1, 172.3, 69.1, 320

II. FIELDS SEARCHED

Minimum Documentation Searched 7	
Classification System	Classification Symbols
U.S. Cl:	536/27; 435/69.1, 172.1, 172.3, and 320

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched 8

Automated patent search; Dialog

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
Y,P	US, A, 5,053,337 (WEINSHANK ET AL) 01 October 1991, see entire document.	1-3,6-9,25-27,77 78
Y,P	US, A, 5,030,570 (BREAKFIELD ET AL) 09 July 1991, see entire document.	1-3,6-9,25-27,77 78

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
09 October 1991	0 1 NOV 1991
International Searching Authority	Signature of Authorized Officer *Diane Moffett for*
ISA/US	Bradley L. Sisson

Form PCT/ISA/210 (second sheet) (Rev.11-87)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	US, A, 4,703,035 (RIVIER ET AL) 27 October 1987, see entire document.	1-3,6-9,25-27 77-78.

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____ , because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers .... , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers _____ , because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

See attached sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

   1-3, 6-9, 25-27, and 77-78

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (supplemental sheet (2) (Rev. 11-87)

LACK OF UNITY

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-3, 6-19, 25-27, and, drawn to nucleic acid sequences, plasmids, vectors, expression systems, nucleic acid probe sequences, cell lines capable of producing recombinant protein, as well as methods directed to the production of said protein, classified in Class 435, subclasses 172.1, 172.3, 69.1, and 320.

Group II, claims 4, 5, and 20, drawn to protein, classified in class 530, subclasses 350 and 412.

Group III, claims 60, 67-69, 72, 75, and 76, drawn to methods of testing and detection, classified in class 435, subclass 6.

Group IV, claims 21-23, drawn to method of screening drugs, classified in class 435, subclass 6.

Group V, claims 24, 28-45, 48-59, 70, 71, and 73, drawn to antisense oligomers and pharmaceutical compositions, classified in class 514, subclass 44.

Group VI, claims 46 and 47, drawn to antibodies, classified in class 530, subclass 387.

Group VII, claims 61-66, drawn to transgenic animal, classified in class 800, subclass 44.

Group VIII, claim 74, drawn to method of using pharmaceutical of Group V, Class 514, subclass 44.

The inventions listed in Groups I-VIII do not meet the requirements for unity of invention for the following reasons: Each Group is drawn to a

distinct invention having its own classification and/or requiring additional searching.

On September 30, 1991, a telephone call was placed to Debra Dugan who spoke on behalf of Representative John P. White. Ms. Dugan indicated at that no papers had been filed and that applicant did not wish to pay any additional fees.